Title: NC03904. Soil Arthropods and Nematodes Associated with No-Tillage Practices in Tobacco

Project Leaders: G. J. House, D. P. Schmitt, and D. M. Jackson

I. Summary of Research:

This project has the following objectives:

A. To investigate the impact of no-tillage and cover cropping practices on the soil arthropod and nematode community structure of tobacco.

B. To compare and quantify predation on tobacco budworm prepupae and pupae occurring in the soil and on the soil surface in conventional and no-tillage systems.

C. To compare and quantify tobacco budworm survival (prepupal and pupal life-stages) under conventional and no-tillage conditions.

Soil arthropods and nematodes were quantified under four tillage treatments: (1) conventional tillage, (2) no-tillage preceded by a winter wheat cover crop, (3) conventional tillage with wheat straw mulch added in spring, and (4) no-tillage with wheat straw mulch added in the spring. All treatments were fall-ridged. Soil arthropod populations were low in both conventional and no-tillage treatments; however, there was a trend for higher numbers of soil surface-dwelling arthropods under no-tillage conditions. Approximately 50% of the soil insects occurring under no-tillage were predators. The combination of no-tillage and a winter cover crop provided a favorable habitat for soil-surface dwelling predaceous arthropods, especially ground beetles and spiders. The addition of wheat straw mulch had a similar effect on insect population dynamics.

Surface wheat straw was also a major factor influencing nematode populations. A trend toward higher nematode populations with straw and no-tillage was observed, but these nematodes were largely non-parasitic forms. Few plant parasitic nematodes were recovered, although there was a statistically non-significant trend for more stunt nematodes in no-tillage with straw treatments.

Experiments examining the influence of tillage practices on tobacco budworm (Heliothis virescens) survival, indicated that budworm pupal mortality was higher under no-tillage than conventional tillage (Table 1). Budworm pupae and pre-pupae were preyed upon by ground beetles, ants, rove beetles and spiders to a greater extent under no-tillage than conventional tillage systems (Table 2).

Penetration of the soil by the prepupal life-stage of the budworm may be more difficult under no-tillage than conventional tillage, which would increase the time of exposure to biotic mortality agents such as predators, and abiotic mortality factors such as body moisture loss.

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Table 1. Influence of tillage practices on tobacco budworm (Heliothis virescens) pupae survival (1986).

Treatment	% Heliothis Recovery *	% Pupal Damage
Conventional Tillage	70	6
Conventional Tillage with Straw	66	9
No-Tillage	55	13
No-Tillage with Straw	56	16

*Intact pupae and moths

Table 2. (1986).

Treatment

Conventic

Conventic with S

No-Tillag

No-Tillag with S

*Predator

**Within

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Table 2. Predation of Heliothis virescens larvae by benefical arthropods* (1986).

Treatment	No. of Predatory Attacks **
Conventional Tillage	14
Conventional Tillage with Straw	10
No-Tillage	20
No-Tillage with Straw	21

*Predators included ground and rove beetles, ants, centipeds, and spiders.

**Within a 4 hour period from 8-12 p.m.

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

VII. Papers Presented at Professional Meetings:

House, G. J., D. M. Jackson, and D. P. Schmitt. Influence of no-tillage practices on soil arthropods and nematodes of tobacco. 32nd Tobacco Workers Conference, January 14, 1987. Baltimore Maryland.

IX. We wish to thank Charles Warrick, Noele Ledford, and Jerry Brust for their excellent technical assistance.

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Title: Biology and Control of Insects That Damage Stored Tobacco

Project Leader: Dennis W. Keever

I. Summary of Research:

This project has the following objectives:

A. To develop biological, chemical, and physical control methods and programs for the cigarette beetle in stored tobacco.

B. To investigate the biology and ecology of stored-tobacco insect species.

C. To investigate how biotic and abiotic elements in tobacco storages affect the trapping of the cigarette beetle.

A. Physical Control (Freezing) of Cigarette Beetles

In a continuation of a 1985 study, a freezing method was developed for the rapid (≤24 hours) disinfestation of the cigarette beetle, Lasioderma serricorne (F.), from tobacco display samples (each ca. 1.6 X 7.1 X 9.4 cm, and 8.6 kg). Previous results had shown that -15°C killed all adults and eggs placed within tobacco samples, but that it required up to 48 hours to do so. Therefore, I examined a colder temperature, -20°C, to determine if an exposure of 24 hours would provide acceptable results.

Freezing at -20°C for 24 hours killed 100% of the eggs, larvae, pupae, and adults placed in the tobacco samples. Pre-conditioning larvae at 10°C for 24 hours did not affect their susceptibility to the cold. Using different ways of placing the samples in the freezer showed that this freezing method is effective only if each sample is exposed on all sides to circulating air.

Using the equation, $Pu = 1 - (1-C)^{1/n*}$), and the methods and results of PHASE II, the proportion insect survival would be estimated at <0.00093, or less than 1 out of 1068 insects ($P<0.05$). Test results have been submitted to APHIS for consideration of this method for phytosanitary certification of tobacco display samples.

*Pu = upper limit on estimated survival rate, C = confidence level and n = number of insects tested. (Ref. Couey and Chew, 1986, J. Econ. Entomol. 79:887-880.)

B. Chemical Control (Fumigation) of Cigarette Beetles

Aluminum and magnesium phosphides were evaluated in tobacco warehouses as cold weather (<4.4°C) fumigants against the cigarette beetle. Determining criteria were (1) mortality of insects placed in tobacco containers during the fumigation/aeration process, (2) the number of beetles caught in light traps during the following warm season, and (3) the rapidity of aeration. An

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unusually warm period began just as fumigation was to be initiated, but we could not delay the fumigation. Therefore, we obtained excellent insect kill as would be expected at these temperatures, but the more important results were those on the patterns of aeration.

Tobacco temperature at the time of fumigation was 9.2°C and max/min temperatures in the warehouses during fumigation and aeration averaged 16.1/9.4°C. All insects placed in the treatment warehouses during fumigation were killed, and trap counts showed that natural cigarette beetle populations were greatly suppressed.

The most serious problem was the slowness of aeration. After 72 hours of aeration, the airspace gas concentration (0.8 ppm) in one warehouse was still too high for legal re-entry (<0.3 ppm). The only gas concentration still too high in the tobacco containers after 72 hours (26. ppm) was in the the warehouse section where the hogsheads were stored. (The other sections held cases only.) All gas concentrations in the airspaces were <0.3 ppm after seven days after aeration. For the aeration we opened only the doors on each end of the warehouse and not the side vents, so under more ventilated conditions aeration might have occurred more rapidly. On the other hand, our temperatures were not very cold, so under more winter-like temperatures the aeration would have been much slower.

C. Monitoring of Insect Pest Species in Farm-Stored Tobacco

In cooperation with P. S. Southern, I monitored populations of the cigarette beetle and the tobacco moth, Ephestia elutella (Hubner), in on-farm storages filled with carry-over tobacco. Packhouses and bulk barns in Yadkin, Guilford, Granville, Pitt, and Edgecombe counties were monitored either weekly or biweekly from May 8 to October 30, 1986 with pheromone traps. This work was a preliminary step to later monitoring efforts, and the results can be stated in several generalizations.

The tobacco moth was commonly found in large numbers, but the cigarette beetle was infrequently found. However, later tests in the laboratory revealed that the trap for the moth was much more effective than was the trap for the beetle. A different beetle trap will be used in future studies. Also trap counts were greater in the eastern part of the state, and were greater in packhouses than in bulk barns.

D. Biological Control (Thuringiensin) for the Cigarette Beetle

Five biocontrol materials were studied for their effects on the cigarette beetle; three formulations of thuringiensin and two species of Bacillus. The thuringiensin formulations, all by Abbott Laboratories, were two 1.5% aqueous solutions (ABG-6211 and ABG-6162A) and one calcium salt formulation in a 10% WP (ABG-6206). All three formulations were applied to an insect rearing medium of ground tobacco at the rates of 15 and 60 ppm. The two bacteria tested were B. cereus and B. popilliae, both at 10 and 10 spores per gram of ground tobacco diet. B. cereus was previously shown to be a naturally occurring pathogen of the cigarette beetle, and although not tested against the cigarette beetle, B. popilliae is a known pathogen of some other Coleoptera.

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Neither of the bacteria at either application rate significantly reduced larval survival. Of the thuringiensin treatments, all three 60 ppm treatments and the ABG-6206 formulation at 15 ppm significantly ($P<0.05$) reduced larval survival (reductions from 61 to 100%). ABG-6206 was the most effective formulation and the 60 ppm rate was more effective than the 15 ppm.

Thuringiensin greatly delayed larval development. Almost all control insects and those on the bacterial treatments began pupation after 8 weeks on the diet, but insects on the ABG-6206 formulation at 60 ppm were still in the 1st instar after eight weeks. Both formulation and application rate were significant factors in delaying larval development.

Thuringiensin also greatly reduced progeny production. While the control diet produced 680 progeny and the bacterial diets produced from 499 to 648 progeny, the three thuringiensin formulations at 60 ppm produced 0 (ABG-6206), 2 (ABG-6162A), and 3 (ABG-6211) progeny.

II. Graduate Students: none

III. Postdoctoral Fellows: none

IV. Publications:

Keever, D. W., M. A. Mullen, J. W. Press, and R. T. Arbogast. 1986. Augmentation of natural enemies for suppressing two major insect pests in stored farmers stock peanuts. Environ. Entomol. 15: 767-770.

Keever, D. W., B. R. Wiseman, and N. W. Widstrom. 1986. Effects of harvesting and drying on Sitophilus zeamais populations in field-infested corn. (Abstract). Phytoparasitica 14:4.

V. Manuscripts Accepted for Review: none

VI. Manuscripts in Review:

Keever, D. W., B. R. Wiseman, N. W. Widstrom. Effects of harvesting and drying on Sitophilus zeamais populations in field-infested corn. Submitted to J. Econ. Entomol.

VII. Papers Presented at Professional Meetings:

Keever, D. W. 1986. Biology of the cigarette beetle, Lasioderma serricorne (F.). North Carolina Pest Control Technicians' School. (Invited paper). Raleigh, North Carolina. January 21-23.

Keever, D. W. B. R. Wiseman, and N. W. Widstrom. 1986. Reduction in maize weevil populations due to threshing and drying of field-infested corn. Southern Corn Improvment Conference. (Invited paper). Memphis, Tennessee. February 12-13.

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Keever, D. W. 1986. Control of insect pests in stored farmers stock peanuts through the augmentation of two entomophagous insect species. Advances in Biological Control session of the Fourth International Working Conference on Stored-Product Protection. (Invited paper). Tel Aviv, Israel. September 21-26.

Keever, D. W., B. R. Wiseman, N. W. Widstrom. 1986. Effects of harvesting and drying on Sitophilus zeamais populations in field-infested corn. Fourth International Working Conference on Stored-Product Protection. (Submitted paper). Tel Aviv, Israel. September 21-26.

IX. Acknowledgments

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Title: NC03699 Pesticide Residues in Tobacco, Tobacco Products, and Main-Stream Smoke

Project Leader: T. J. Sheets

I. Summary of Research:

Residues of flumetralin on Flue-Cured Tobacco and in Tobacco Soils. Experiments were conducted at the Border Belt Research Station (Whiteville) and the Upper Piedmont Research Station (Reidsville). In a continuing study to determine residues of flumetralin (Prime +) in flue-cured tobacco and in soils from treated plots. Before bedding in preparation for transplanting, two plots in each of four replication were sprayed with 1.7 kg/ha of isopropalin (Paarlan) as a broadcast treatment, and the herbicide was incorporated by double disking. The tobacco was topped and a contact sucker control agent was applied to all plots. Seven days later flumetralin was applied to one isopropalin-treated plot and to one plot not receiving isopropalin at a rate of 1.35 kg/ha. Residues of flumetralin in cured tobacco averaged 0.8, 0.5, 0.5, and 0.2 ppm from harvests 1 to 4, respectively at the Upper Piedmont Research Station. Samples of tobacco from the Border Belt Research Station were lost. Residues of flumetralin in soil were about the same regardless of the treatment and location. Levels varied between 0.28 and 0.34 ppm.

EBDC and ETU Residues on Flue-Cured Tobacco. Study of EBDC and ETU residues on Flue-cured tobacco was continued in 1985 at the Central Crops Research Station (Clayton). Maneb and mancozeb were applied at a rate of 0.9 kg/380 L at 5 or 7-day spraying intervals beginning May 30, 1985 and ending July 15, 1985. With a 5-day spray interval, residues of EBDC and ETU in samples composited over stalk positions averaged 28 and 0.06 ppm, respectively, for maneb-treated plots compared to 48 and 0.25 ppm, respectively, for mancozeb-treated plots. When a spray interval of 7 days was used, EBDC and ETU levels were 42 and 0.19 ppm from mancozeb-treated plots. For a reduced spray strategy with 5-day intervals and four applications of mancozeb beginning June 3 and ending June 17, residues were 18 and <0.05 ppm of EBDC and ETU, respectively.

MH Residues in 1985 Tobacco Products. MH residues in 1985 tobacco products were 52, 10, 30, 18, and 23 ppm for cigarettes, cigars, pipe tobacco, chewing tobacco, and snuff, respectively. The 1985 residue for cigarettes (52 ppm) was lower than that for 1984 (66 ppm). MH residues in other products have not changed appreciably since 1975.

Residues of Chlorinated Hydrocarbon Insecticides in 1985 Tobacco Products. Average levels of total DDT residues (TDE plus DDT) were 0.19. 0.81, 0.26, 0.28, and <0.12 ppm, respectively, for 1985 cigarettes, cigars, pipe tobacco, chewing tobacco, and snuff. The average for total DDT residues dropped between 1984 and 1985 for cigars and remained about the same for other products. Residues of toxaphene, dieldrin, and endrin were below the lowest detectable limits in all tobacco products.

Aldicarb Residues in 1985 Tobacco Products. Total aldicarb residues (aldicarb, aldicarb sulfoxide, and aldicarb sulfone) were below the lowest detectable limit of 0.2 ppm in all 1985 tobacco products analyzed.

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Chlorine Content of 1985 Flue-Cured and Burley Tobacco. The chlorine content in composite samples of flue-cured tobacco ranged from a low of 0.44 to a high of 0.93% and averaged 0.65% over all belts and stalk positions. Averages were highest for the Eastern and Border Belts and lowest for the Georgia-Florida and Old Belts. The composite sample with the highest value (0.93%) was from the Georgia-Florida Belt.

The average chlorine content for 24 composite samples of burley was 0.72%. Values ranged from 0.42 to 1.06%. All composite samples from marketing Area D exceeded 0.80% with an area average of 0.93. Averages for Areas A, B, and C were 0.57, 0.74, and 0.62%, respectively.

Effects of Soil-Applied Picloram and Dicamba on Flue-Cured Tobacco. Field experiments were conducted at three locations in 1985 to compare the effects of picloram, dicamba, triclopyr, and 2,4-D on flue-cured tobacco. Of special interest was the comparative effects of picloram and dicamba on leaves and roots. Rates of application were 0.025, 0.1, 0.4, and 1.6 g ai/ha for picloram, 0.1, 0.4, 1.6, and 6.4 g ai/ha for dicamba, 0.4, 1.6, 6.4, and 25.6 g ai/ha for triclopyr, and 1.6, 6.4, 25.6, and 102 g ai/ha for 2,4-D. Dicamba, triclopyr, and 2,4-D did not cause discernable effects in 1985 at any of the rates used. Plots that received triclopyr in 1985 were treated with picloram in 1986 at 0.025, 0.1, 0.4, and 1.6 g ai/ha. The 2,4-D plots from 1985 were treated with dicamba at 6.4, 25.6, 102, and 410 g ai/ha in 1986. The 1985 picloram and dicamba plots were not retreated. Tobacco (McNair 944) was grown in all plots both years according to standard cultural practices. The herbicides were applied as a spray band in a furrow 6 to 10 cm deep and 8 to 12 cm to the side of the plants within 7 days after transplanting. Care was taken to prevent spray drift onto the plants. The furrows were closed after spraying. Injury ratings were made three times at 2-week intervals during the growing season beginning about 6 weeks after treatment. Near the time of the first harvest, four soil cores 5 cm in diameter and 15 cm deep were removed from sites in the center of the row, each one mid-way between two plants. In 1986, root samples were taken from control plots and from those treated in 1986. Roots were separated from soil using a nematode elutriator, and total length, average diameter, total volume, and total surface area determined by use of a video-image digitizer interfaced with an Apple computer. Tobacco leaves were harvested and cured, and yields and grade of cured leaf determined.

Slight symptoms were observed on tobacco growing on plots that received 0.025 g/ha of picloram in both years, and symptoms increased to severe at the 1.6 g/ha rate. Although dicamba did not produce significant symptoms at 6.4 g/ha in 1985, the same rate in 1986 caused mild symptoms at one location; and injury increased to severe at 410 g/ha. About 60 to 70 times as much dicamba was required to cause the same degree of injury symptoms as picloram and about 150 to 200 times as much to cause similar yield reductions. Symptoms caused by the two herbicides were usually indistinguishable. Root measurements were highly variable; however, significant changes in root length, volume, diameter, and surface area appeared to occur only after leaves of plants were severely malformed. Yield of cured leaves in 1986 was significantly reduced from 0.4 g/ha of picloram and severely reduced from 1.6 g/ha. Dicamba at 102 g/ha

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significantly reduced yield and 410 g/ha severely reduced yield. The grade index was significantly reduced by 1.6 g/ha of picloram and at 410 g/ha of dicamba. Mild symptoms were consistently observed at all locations in 1986 from 1.6 g/ha of picloram applied in 1985. The studies showed that mild symptoms (slight to moderate leaf cupping) caused by picloram or dicamba do not necessarily indicate that yield or quality of flue-cured tobaccos will be reduced.

II. Graduate Students:

Powell, Kevin E., MS, Toxicology
Thesis title: Metabolic Fate of ^{14}C-Maleic Hydrazide in Tobacco Plants.

III. Postdoctoral Fellows: None

IV. Publications:

Sheets, T. J. and B. H. Harrell, Jr. 1986. Effects of Low Levels of Picloram on Flue-Cured Tobacco. North Carolina Agri. Res. Serv. Tech. Bull. 280. 23 p.

Leidy, R. B., T. J. Sheets, and L. A. Nelson. 1986. Residues of fluvalinate and permethrin on flue-cured tobacco. Beitr. Tabakforsch. 13:191-203.

Meyer, Susan A., T. J. Sheets, and H. Seltmann. 1987. Maleic hydrazide residues in tobacco and their toxicological implications. Rev. Environ. Contam. Toxicol. 98:43-60.

V. Manuscripts Accepted for Publication:

Atwood, S. T., T. J. Sheets, T. B. Button, and R. B. Leidy. Stability of selected pesticide formulations and combinations in aqueous media. J. Agri. Food Chem.

VI. Manuscripts in Review:

Seltmann, H. and T. J. Sheets. Sucker control and maleic hydrazide residues after simulated rainfall and MH reapplication. Tob. Sci.

Leidy, R. B., T. J. Sheets, and C. G. Wright. DDVP residues in air following application to a tobacco storage warehouse. Environ. Monit. Assess.

VII. Papers Presented at Professional Meetings.

Residues of Prime + on Flue-Cured Tobacco. R. B. Leidy, T. J. Sheets, and H. Seltmann. Tobacco Workers Conference, Baltimore, MD, January 12-15, 1987.

Effects of Soil-Applied Picloram and Dicamba on Flue-Cured Tobacco. T. J. Sheets and A. D. Worsham, Southern Weed Sci. Soc., Orlando, FL, January 19-21, 1987.

VIII. Graduate Student Theses Completed During Reporting Period: None

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Title: NC 03963 GENETIC INVESTIGATION OF NICOTIANA POPULATIONS

Project Leaders: E. A. Wernsman and D. F. Matzinger

I. Summary of Research:

A. Restricted Selection Index

In most breeding programs, when selection is carried out to modify a particular trait, changes occur in other traits because of correlated effects. These correlated effects can occur because of genetic linkage or pleiotropy. Correlated effects are useful if the direction of the correlation is the same as the direction of the desired response. On the other hand, if the genetic correlation is opposite to the desired change in mean performance, the correlations are a major barrier to improving the organism for multiple traits.

One of the genetic techniques to modify jointly several correlated traits is the use of selection indexes. A special type of index is a restricted selection index where the goal is to maximize one trait while holding one or more correlated traits at the population mean. An experiment is in progress in an attempt to increase total alkaloids (TA) in the population while maintaining yield at the level of the initial population.

Three cycles of restricted index selection have been conducted beginning with the F_2 generation of a cross between NC13 and McNair 135. NC13 is a high yielding cultivar and McNair 135 has moderate yield. Both cultivars have relatively high TA levels and they have generated among the lowest levels of total particulate matter of released cultivars.

The genetic correlation between yield and TA in the base population was -0.67. Predictions of selection gains indicated that selection solely for increased %TA in this population would be expected to increase TA 4.1% per cycle, but that a correlated decrease in yield would be 1.9%. Predictions for the restricted index were a 2.4% increase in TA with no change in yield.

The original parents and three cycles of selection were evaluated at 2 locations and 2 years with 10 replications in each experiment (Table 1). Significant differences existed among the cycle means for TA with an average increase of 2.5% of the mean per cycle. There were no significant differences among the cycles for yield, with an average decrease in yield of 0.5% per cycle. No changes occurred in total particulate matter, as measured by PMI, during the 3 cycles of selection. The PMI/TA ratio decreased an average of 2.16% per cycle; however, these differences were not significant.

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Table 1. Mean performance of parents and selected cycles.

Entries	TA %	Yield lb/A	PMI mg/g	PMI/TA
NC13	2.69	3500	191	9.1
McN 135	2.72	3198	190	8.2
C0	2.64	3411	189	8.4
C1	2.68	3369	190	8.5
C2	2.85	3304	190	7.9
C3	2.81	3378	190	8.0
Sig.	*	ns	ns	ns
Response, %/Cycle	2.5	-0.5	0.16	-2.16

* Cycles different at P=.05
ns Cycles not different at P=.05

The results from this study confirm that one can use a restricted selection index to increase TA and negate the normal decrease in yield when increasing TA. Additional generations of selection will be necessary to determine if this index will also lead to significant reductions in the PMI/TA ratio.

B. Nuclear DNA Investigations (with Lino Fragoso)

Studies have continued on isolation of nuclear genes of proteins that are transported into the mitochondria in order to identify and characterize their transit sequences. The approach was to use complementary DNA clones from other organisms as a probe. Porcine and human clones to ornithine transcarbamylase were used to screen a complementary DNA library cloned into the lambda vector gt10. A clone containing an insert of 1.6 kilobase pairs of N. tabacum DNA was isolated from the library. A sequence homology search indicated 70-80% homology to human ornithine transcarbamylase. However, this tobacco clone was missing the first 300 bases of the gene, which is the region where the transit sequence is located. We are using these complementary DNA clones to screen the genomic DNA libraries to isolate the genomic DNA clone containing the gene for ornithine transcarbamylase.

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C. The Haploid Sporophyte as the Selection Unit in Tobacco Breeding

The Nicotiana africana method of haploid production has been refined sufficiently to permit the production of large haploid populations from desired sources. Chromosome doubling of haploid sporophytes from egg nuclei results in homozygous lines whose agronomic performance compares favorably with conventionally-inbred genotypes. Furthermore, the procedure saves 3 to 4 years in cultivar development. The limiting factor in commercial utilization of the procedure is chromosome doubling. Colchicine treatments give poor frequencies of chromosome doubling; in vitro tissue culture methods are highly reliable for chromosome doubling but are very laborious. Because random inbred lines have a low probability of being useful as new cultivars via any plant breeding procedure, chromosome doubling a large array of random haploid plants requires large labor expenditures in doubling lines that will be shown later to be of little value. Hence, we have directed much effort to methods for identifiying individual haploid plants that possess desirable genotypes prior to chromosome doubling. Chromosome doubling using in vitro procedures is then restricted only to those genotypes which possess merit. This has proven to be highly reliable for simply-inherited disease resistance mechanisms such as resistance to TMV, root-knot nematodes, wildfire and potato virus Y. Investigations have been pursued to extend the procedure to important quantitatively-inherited traits such as leaf yields, plant form, and leaf concentrations of nicotine and sugars.

To successfully conduct mass selection among haploid sporophytes, three criteria must be met; 1) the capacity to produce large haploid populations in outdoor plant beds is needed; 2) a high correlation of performance of haploid and doubled haploid genotypes is required; 3) haploid plants identified as being superior must be chromosome doubled by in vitro methodologies.

Nine burley cultivars were crossed with pollen of Nicotiana africana and resulting seed were sown at two dates in outdoor plant beds with a greenhouse seeding used as a control. Although haploid survival in plant beds was poor compared to the greenhouse, 0.7 plants per capsule were recovered and would be sufficient for the production of moderate-sized populations.

Agronomic performance and chemical composition of cured leaf of nine genotypes in haploid and diploid conditions were measured in six replications of a split-plot design grown at the Central Crops Research Station in 1984 and 1985. Data were collected on green leaf yield, cured leaf yield, plant height, leaf number, days to flower, and percentages of total alkaloids as nicotine and reducing sugars in cured leaf. Although

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environment x ploidy interaction was highly significant, rank order changes in means of genotypes across the two polidy levels were not great. Correlations of genotypic performance in haploid and diploid conditions were positive and significantly different from zero for all characters except days to flower and reducing sugars in cured leaf as shown below.

Character	Correlation
Green Leaf Yield	0.89**
Cured Leaf Yield	0.84**
Total Alkaloids	0.79
Reducing Sugars	0.37**
Leaf Number	0.90**
Plant Height	0.78
Days to Flower	0.56

**Significantly different from zero at P=.01

These data suggest that mass selection for quantitative traits in haploid populations may be an effective means of tobacco improvement, and would permit labor expended in chromosome doubling to be concentrated on genotypes that have merit.

In vitro cultures of leaf tissue from field-grown plants are frequently contaminated by nonpathogenic bacteria and fungi in vascular tissues. Tests of the efficacy of antibiotic compounds to supress and control these microbial contaminations have been investigated, but results have not been favorable. Alternate methods of maintaining aseptic cultural conditions are needed.

II. Graduate Students:

Bland, Molly M., Ph.D. Candidate, Genetics. Molecular genetics of tobacco.

Corbin, Thomas, C., Ph.D. Candidate, Crop Science. A comprehensive tobacco improvement program utilizing parthenogenic haploids.

Wilhite, Faye M., Ph.D. Candidate, Crop Science. Mass selection for quantitatively-inherited traits in tobacco haploid populations.

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Witherspoon, William David, Jr., Ph.D. Candidate, Crop Science. Utilization of the haploid sporophyte as the selection unit in plant breeding.

Yung, Carl H., Ph.D. Candidate, Crop Science. DNA amplification in anther culture-derived haploids and its relationship to changes in tobacco metabolism.

III. Postdoctoral Fellows:

Fragoso, Lino. Cloning of Nicotiana DNA.

IV. Publications:

Bland, M. M., C. S. Levings III, and D. F. Matzinger. 1986. The tobacco mitochondrial ATPase subunit 9 gene is closely linked to an open reading frame for a ribosomal protein. Mol. Gen. Genet. 204:8-16.

Bowman, D. T., E. A. Wernsman, T. C. Corbin, and A. G. Tart. 1986. Stability of flue-cured tobacco cultivars for yield and quality. Tob. Sci. 30:16-19.

Rufty, Rebeca C., E. A. Wernsman, and G. V. Gooding, Jr. 1987. Evaluation of tobacco haploids and doubled haploids for resistance to tobacco mosaic virus, Meloidogyne incognita and Pseudomonas syringae pv. tabaci using detached leaves. Phytopathology 77:60-62.

V. Manuscripts Accepted for Publication:

Wernsman, E. A. and R. C. Rufty. 1987. Tobacco. In Handbook of Plant Breeding II, W. F. Fehr (ed.). Macmillan Publishing Co. Inc. New York, NY. (in press)

VI. Manuscripts in Review:

Rufty, Rebeca C., E. A. Wernsman, and G. V. Gooding, Jr. 1987. Inheritance of resistance to tobacco etch virus in Nicotiana tabacum L. cultivar Havana 307.

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VII. Papers Presented at Professional Meetings:

Bland, M. M., D. F. Matzinger, and C. S. Levings, III. Variation in mitochondrial transcription patterns among Nicotiana species, CMS and restored lines. Internat. Workshop on Higher Plant Mitochondrial DNA. Oct. 19-24, 1986. Arlie, VA.

Matzinger, D. F., E. A. Wernsman, and W. W. Weeks. Restricted index selection for yield and total alkaloids. 32nd Tobacco Workers Conf. Jan. 12-15, 1987. Baltimore, MD.

Reed, S. M. and E. A. Wernsman. Use of irradiated pollen in Nicotiana tabacum. Amer. Soc. Agron. Meetings. Dec. 1-5, 1986. New Orleans, LA.

Rufty, R. C., E. A. Wernsman, and C. E. Main. Evaluation of tobacco germ plasm for resistance to blue mold in Puerto Rico. 32nd Tobacco Workers Conf. Jan. 12-15, 1987. Baltimore, MD.

Witherspoon, W. D., Jr. and E. A. Wernsman. In vitro anther culture as an aid to interspecific gene transfer. Amer. Soc. Agron. Meetings. Dec. 1-5, 1986. New Orleans, LA.

Witherspoon, W. D., Jr. and E. A. Wernsman. The haploid sporophyte as the selection unit in tobacco breeding. 32nd Tobacco Workers Conf. Jan. 12-15, 1987. Baltimore, MD.

Wernsman, E. A., W. D. Witherspoon, Jr., and G. V. Gooding, Jr. Nicotiana africana as a source of virus resistance for tobacco improvement. 32nd Tobacco Workers Conf. Jan. 12-15, 1987. Baltimore, MD.

VIII. Graduate Student Theses Completed During Reporting Period:

Witherspoon, William David, Jr. Utilization of the haploid sporophyte as the selection unit in tobacco breeding. Ph.D. Thesis, 1987.

IX. Acknowledgments:

This work was supported in part by the North Carolina Tobacco Foundation, Inc. and R. J. Reynolds Tobacco Company.

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Title: NC 05590 Characterization of cis-acting sequence determinants for the regulation of gene expression in plants.

Project Leader: Mark A. Conkling

I. Summary of Research:

The objectives of this research program is to characterize DNA sequences which act in cis to regulate organ-specific gene expression in tobacco. To do this we are cloning genes which are expressed in organ-specific fashions. These will be reintroduced into tobacco cells. Following plant regeneration, the organ-specific expression of the input gene will be determined. Those genes which exhibit the predicted organ-specific expression must contain sequences regulating that expression and will be characterized further.

The organ-specific cDNA clones had been isolated on the basis of steady state mRNA levels. These could result from either transcriptional or post-transcriptional regulation. To distinguish these, transcription rates were measured in isolated nuclei. The data are consistant with regulation at the transcriptional level.

We have cloned and characterized the genes encoding the organ-specific cDNA's. A genomic library of 5×10^6 recombinants was constructed in a lambda vector and screened with organ-specific cDNA probes. Multiple independent clones were isolated for each cDNA. These genomic clones have been restriction mapped using a novel indirect mapping protocol developed in my laboratory. The transcribed regions have been mapped. We are currently mapping the site of transcription initiation by S1 and RNA::DNA heteroduplex analysis.

We have developed a binary vector system to facilitate the Agrobacterium-mediated transformation of tobacco cells. This vector contains a polylinker cloning site adjacent to a reporter gene. Cis-acting sequences will be cloned into the polylinker and used to regulated the expression of the reporter gene. These vectors have been used to produce transgenic plants.

II. Graduate students:

Yuri Yamamoto (Genetics)

Judy Jakobek (Botany)

III. Postdoctoral fellows:

Dr. Donald DeJong-Visiting scientist from the Tobacco Research Labroatory, USDA ARS, Oxford, NC.
January 1, 1986 - September 30, 1986.

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IX. Acknowledgemnts:

I wish to thank Ms. Carolyn Faucett for her excellent technical assistance. I also wish to acknowledge Dr. Judy Thomas and the staff of the Southeastern Plant Environmental Laboratories for their help in growing tissue under controlled environmental conditions.

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Title: NC03896 Cell Culture Techniques for the Development of Disease Resistance in Tobacco

Project Leader: Margaret E. Daub

I. Summary of Research:

This project has three broad objectives:

1. To investigate the potential of somaclonal variation for generating novel, disease-resistant genotypes.

2. To develop protoplast fusion techniques for transferring naturally occurring resistance genes from wild Nicotiana species into tobacco cultivars.

3. To investigate the molecular mechanism of resistance in one host-pathogen system (the tobacco/Cercospora nicotianae system) in order to identify genes for resistance which may be important for tobacco improvement.

A. Somaclonal Variation.

One of the most promising areas in the use of tissue culture for the development of novel crop genotypes is the observation that the process of growing cells in culture can generate stable, heritable variation which is useful in crop improvement. This variation has been termed somaclonal variation, and has been demonstrated in a number of crops including sugar cane, potatoes, and tomatoes, and for a number of different traits including both disease resistance and agronomic traits. The purpose of this work is to investigate the usefulness of somaclonal variation for developing high quality tobacco cultivars with increased levels of disease resistance.

We have isolated mesophyll protoplasts from several tobacco cultivars (Coker 319, NC 2326, Speight G-28, K-399) which have desirable quality characteristics but lack high levels of resistance to a number of tobacco diseases and pathogens including black shank, Granville Wilt, root knot nematodes and tobacco mosaic virus (TMV). The protoplasts were induced to form callus and were maintained as callus for several months in order to increase the probability of inducing variability. Plants were then regenerated from these calli, and transferred to soil in the greenhouse. These plants were allowed to flower, and selfed seed was collected from each plant. This latter step is required in order to ensure that all variation seen among the plants is limited to changes which are passed through a seed cycle.

So far, progeny of over 400 somaclones of Coker 319 and NC 2326 have been evaluated in the field and greenhouse for resistance to black shank, Granville wilt, TMV, and root knot nematodes. Large variations have been found in resistance to

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black shank and Granville wilt with most lines showing equivalent or lower levels of resistance to these diseases as compared to the parent cultivars. Some somaclone progeny of NC 2326 have shown increased levels of black shank resistance in field trials over two years. These lines and others currently being generated will be tested again in the field and greenhouse in 1987.

The major problem with our somaclone studies has been the amount of variation seen in field and greenhouse tests for disease resistance. In a single disease nursery in the field, for example, variations in disease levels in replications of a single cultivar often exceed the differences seen among cultivars with significantly different levels of disease resistance. To alleviate this problem, we are now developing infectivity titration methods which should allow us to accurately detect differences in disease resistance among somaclones of a particular variety. In this work we are using somaclone progeny of Speight G-28 and K-399 and measuring differences in levels of Granville wilt resistance. Lines showing variation based on these more accurate inoculation techniques will then be further evaluated in the field.

B. Transfer of Resistance Genes from Nicotiana repanda to Nicotiana tabacum by Protoplast Fusion.

The wild species, Nicotiana repanda, has been shown to possess resistance to more tobacco diseases than any other Nicotiana species. Its resistance to three root knot nematode species and to TMV are of particular importance for tobacco improvement. Attempts to produce hybrids of N. repanda and N. tabacum by conventional breeding methods have been unsuccessful. To overcome the obstacles to sexual hybridization, somatic hybridization was attempted by fusion of protoplasts and culturing of somatic cells.

In order to identify any somatic hybrids produced by protoplast fusion, we used protoplasts of N. tabacum plants homozygous for the semi-dominant nuclear albino mutation (Su/Su). Since our N. repanda plants lacked this gene, we could select for hybrid fusions by looking for light green shoots in contrast to the albino and dark green shoots of the parents. Protoplasts of the two species were released by overnight incubation in 1% Cellulysin and 0.2% Macerase, dissolved in protoplast buffer with 0.5 M mannitol. After isolation the protoplasts of the two species were mixed and fused by the addition of polyethylene glycol (PEG) (MW 8000). After washing out the PEG, the cells were cultured in liquid medium with decreasing concentrations of mannitol for one month, then cell clumps were transferred to regeneration medium without mannitol. When shoots were produced, albino and dark green regenerants were discarded and light green ones transferred to rooting medium. When light green plants were 2-3" high and had a good root system they were transferred to pots in the greenhouse. Optimum growing conditions for these

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plants proved to be in a greenhouse at 80°C, shaded with brown paper and watered sparingly. About 11% of light green plants transferred to soil did not survive.

Out of 48 fusion experiments, plants were regenerated only four times, probably due to the toxicity of PEG. In these four experiments, light green plants were regenerated at frequencies of six, eleven, three, and two percent, for a total of 126 plants. These putative hybrids have been evaluated for hybridity by phenotype, cytogenetics, disease resistance, and isozyme analysis. Some of the plants had 47 chromosomes and looked like N. tabacum. Others had 86-95 chromosomes and appeared phenotypically to be hybrids. However, we have no evidence of hybridity in any of these lines. All 126 lines are susceptible to TMV. Twenty-seven lines were evaluated for resistance to Meloidogyne javanica; all were susceptible. Twenty-nine of the plants have flowered, and all have typical N. tabacum flowers. All 126 have also been evaluated by isozyme analysis. Twenty-six enzymes systems were tested. Most showed no difference between the "hybrids" and the N. tabacum parent. With one enzyme system, phosphohexose isomerase, approximately one half of the "hybrids" had a pattern which was different from either parent. The basis for this difference is currently unknown, but does not appear to be due to hybridity. We have concluded that the reversion rate of our albino marker is too high to be useful in fusion experiments between species such as N. tabacum and N. repanda where low hybrid survival is expected.

We are currently trying to overcome some of the problems encountered in this study. We are now using a hybrid between N. repanda and N. sylvestris as one parent. N. sylvestris shows homology with both N. tabacum and N. repanda, and use of this hybrid may improve hybrid viability. Also, we are now using plant transformation vectors to transform the parents for antibiotic resistance, and will use antibiotic resistance as a selection system. Such as selection system should allow us to select hybrids at an early stage of protoplast regeneration and should overcome many of the problems associated with our albino selection system.

C. Mechanisms of Resistance to the Phytotoxin Cercosporin Produced by Members of the Genus Cercospora.

The purpose of this work is to determine the genetic and biochemical basis of resistance to cercosporin, a toxin produced by fungal plant pathogens in the genus Cercospora. Previous work has failed to identify sources of cercosporin resistance in plants, and attempts to select tobacco protoplasts and cell cultures for resistance to cercosporin in vitro have not been successful. Thus we are studying the resistance mechanisms of the fungus with the hope that the information obtained will be useful in developing cercosporin-resistant plants.

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Our studies suggest that the cell wall is the primary site of cercosporin resistance in Cercospora fungi. Protoplasts of C. nicotianae are considerably more sensitive to cercosporin than is fungal mycelium. As the protoplasts start to regenerate a cell wall, they regain their original level of resistance. Protoplasts of the cercosporin-sensitive fungus Neurospora crassa show the exact same response to cercosporin as do those from C. nicotianae, but as the N. crassa protoplasts start to regenerate a cell wall, they do not become any more resistant. The differences between the Cercospora and Neurospora cell wall which are responsible for the differences in resistance are not known at this time. Cercospora and Neurospora show the same rate of regeneration of mannose and β-glucans, and both lack galactose. Chitin, however, is laid down considerably earlier in Cercospora as compared to Neurospora. We do not believe that chitin plays any role in resistance to cercosporin, for both fungi have chitin-containing walls. The difference in timing of chitin deposition, however, indicates that there are differences in the walls of these fungi.

We have also looked at chemical quenchers which may be involved in quenching of the active oxygen species (superoxide and singlet oxygen) which are generated by cercosporin and are the primary means by which cercosporin damages cells. Carotenoids, which quench singlet oxygen, are very important. Cercospora species produce high concentrations of β-carotene, and carotenoid-deficient mutants are more sensitive to the toxin than are wild-type strains. Antioxidants and oxidative enzymes such as superoxide dismutase, catalase, and peroxidase do not appear to be important.

Our present efforts are directed at obtaining mutants which can be used to further characterize resistance mechanisms. These efforts are twofold: 1) to isolate cercosporin-resistant Neurospora mutants, and 2) to develop techniques for transforming Neurospora with a Cercospora DNA library to obtain cercosporin-resistant transformants.

IIA. Graduate Students:

Kimberly D. Gwinn, PhD Candidate in Plant Pathology. Thesis Topic: "Resistance to Cercosporin in the genus Cercospora".

John Marhoul, MS Candidate in Plant Pathology. Thesis topic: "Somaclonal Variation for Granville Wilt Resistance in Tobacco".

IIB. Special Students:

Angela Frazier. Research Area: Somaclonal Variation in Tobacco.

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III. Postdoctoral Fellow:

Dr. Anne E. Jenns. Research Area: Protoplast Fusion in Tobacco.

IV. Publications:

Daub, M. E. 1986. Tissue culture and the selection of resistance to pathogens. Ann. Rev. Phytopathol. 24:159-186.

V. Manuscripts Accepted for Publication:

Daub, M. E. 1987. The fungal photosensitizer cercosporin and its role in plant disease. In: J. R. Heitz and K. R. Downum, eds. Light-Activated Pesticides. American Chemical Society Books, Washington, D.C. (In press).

VI. Manuscripts in Review:

Daub, M. E. Resistance of fungi to the photosensitizing toxin, cercosporin. (Submitted to Phytopathology).

Gwinn, K. D., and M. E. Daub. Regenerating protoplasts from Cercospora and Neurospora differ in their response to cercosporin. (Submitted to Phytopathology).

Gwinn, K. D., M. E. Daub, and P.-Y. Huang. Cell surface changes in regenerating protoplasts of Cercospora nicotianae and Neurospora crassa. (In departmental review, intended for publication in Experimental Mycology).

VII. Papers Presented at Professional Meetings:

Gwinn, K. D., and M. E. Daub. 1986. Cell wall regeneration does not affect the sensitivity of Neurospora protoplasts to cercosporin. Phytopathology 76:1080-1081. (Abstract)

Daub, M. E. 1987. Somaclonal variation for the development of disease resistance in tobacco. Talk presented at the 32nd Tobacco Worker's Conference.

VIII. Graduate Student Theses Completed During Reporting Period:

Gwinn, K. D. 1987. Resistance to Cercosporin in the Genus Cercospora.

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IX. Acknowledgements:

The excellent technical assistance of Mr. D. Wayne Pollard is greatly appreciated. This work has been supported by grants from R. J. Reynolds Corporation, The North Carolina Tobacco Foundation, The North Carolina Biotechnology Center, and the USDA Competitive Grants Program.

2000270084

Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Title: NC 03794 Virus Diseases of Solanaceous Crops

Project Leader: G. V. Gooding, Jr.

I. Summary of Research:

Seven naturally occurring strains of potato virus Y (PVY) from around the world were purified and chemically characterized. The primary difference among the strains was that capsid and inclusion body protein from the U.S.-MM strain differed from the other strains. Such information is basic to understanding virus strains and their biological behavior. There was no difference in the incidence of PVY between insecticide treated or nontreated fields of flue cured tobacco. The insecticide treated fields received carbofuran preplant and weekly applications of acephate. Colonizing aphids were not observed in treated fields. This information supports the recommendations against use of insecticides to control aphid-transmitted viruses on tobacco in North Carolina. In cooperation with plant breeders, two lines of tobacco tolerant to blue mold and not more susceptible to PVY than commercial tobacco cultivars were chosen for release. These lines do not have the limitation of linked PVY tolerance-blue mold susceptibility as was found with tobacco line, Virgin A mutant. A three-year evaluation of commercial cultivars of burley tobacco for tolerance to tobacco vein mottling and tobacco etch viruses was completed. This information will be of value in control recommendations for these viruses.

II. Graduate Student:

Co-Chairman: Richard F. Allison

III. Publications:

Gooding, G. V., Jr. 1986. Tobacco mosaic virus. pp. 133-152 in: The Rod-Shaped Viruses. Van Regenmortel and Frankel-Conrat, eds. Plenum Publ. Corp., NY.

Miner, G. S., Gooding, G. V., Jr., and Moore, J. M. 1986. Control of inoculum sources of tobacco mosaic virus on tobacco plant beds. Tobacco Sci. 30:25-29.

Park, E. K., and Gooding, G. V., Jr. 1986. Tolerance of Nicotiana tabacum cultivars Dixie Bright 244-2, McNair 30, and Golden Stock Penish to strains of potato virus Y. Korean J. Plant Path. 2:12-16.

IV. Manuscripts Accepted for Publication:

Gooding, G. V., Jr., and Rufty, R. C. Distribution, incidence, and strains of viruses in burley tobacco in North Carolina. Plant Disease.

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Rufty, R. C., Wernsman, E. A., and Gooding, G. V., Jr. Evaluation of tobacco haploids and double-haploids for resistance to tobacco mosaic virus, Meloidogyne incognita, and Pseudomonas syringae pv. tabaci using detached leaves. Phytopathology.

VI. Manuscripts in Review:

Rufty, R. C., Wernsman, E. A., and Gooding, G. V., Jr. Inheritance of resistance to tobacco etch virus in Nicotiana tabacum L. cultivar Havana 307. Phytopathology.

VII. Papers Presented at Professional Meetings:

Gooding, G. V., Jr. 1987. Epideiology and control of tobacco mosaic. The 32nd Tob. Workers Conf., Baltimore, MD.

Gooding, G. V., Jr. 1987. Control of severe strains of potato virus Y using cross protection. The 32nd Tob. Workers Conf., Baltimore, MD.

Gooding, G. V., Jr. 1987. Tomato spotted wilt virus in tobacco. The 32nd Tob. Workers Conf., Baltimore, MD.

Wernsman, E. A., Witherspoon, W. D., Jr., and Gooding, G. V., Jr. 1987. Nicotiana africana as a source of virus resistance for tobacco improvement. The 32nd Tob. Workers Conf., Baltimore, MD.

VIII. Acknowledgements:

Appreciation is extended to J. B. Young for excellent technical assistance.

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Title: NC 03773 Soilborne Diseases of Tobacco

Project Leader: H. D. Shew

I. Summary of Research

Greenhouse and field trials with the black root rot pathogen, Thielaviopsis basicola, (Tb) were completed. Soil was infected with known numbers of Tb propagules and 7 cultivars representing different sources and levels of resistance were transplanted into the soil. A set of differential cultivars was selected for future screening for races of the pathogen. In a test using different isolates of Tb, differences in the level of general virulence but not specific virulence was observed. Data are summarized in Tables 1 and 2.

Five on-farm tests were established to determine the effect of soil fumigation and host resistance to Tb on disease losses. Initial populations of Tb ranged from 90 to 700 colony forming units (CFU) per gram of soil. Fumigation reduced the population as much as 86% three weeks after application, and populations in fumigated rows remained low throughout the season. In nonfumigated rows, populations of Tb increased in the root zone of susceptible and low to moderate resistance cultivars, but remained the same or declined in high resistance cultivars. Fumigation resulted in a yield increase, above and beyond that attributable to control of black root rot, of 243 lbs/acre in one field and 409 lbs/acre in another field. Data are summarized in Tables 3-5.

Investigations continued on the biology of the Rhizoctonia leaf spot disease of tobacco. A host range study was completed in which twenty plant species were inoculated. Tobacco was the only plant with significant levels of disease. The only plants attacked (basidiospore inoculum) in addition to tobacco were eggplant and sugar beet. Soybean, corn, peanut, cotton, and bean were not attacked.

Misting regime and temperature effects on lesion number and lesion expansion were determined for flue-cured tobacco exposed to basidiospores of the leaf spot pathogen. Optimum misting regime was continuous mist. Optimum temperature for lesion number (infection) was 26-20 C, day:night, whereas optimum temperature for lesion expansion was 30-24 C. Temperatures above 30 were inhibitory for all stages in pathogenesis. Data are summarized in Tables 6 and 7.

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Table 1. Effect of isolate on root rot severity.

Isolate origin	Severity[x,y]
Buncombe	1.92 a
Madison	1.93 ab
Yancey	2.10 b

[x]Severity on a 1-5 scale with 1=Healthy and 5= completely necrotic root system.

[y]Value is average across all cultivars and all inoculum densities.

Table 2. Response of burley cultivars and Nicotiana debneyi to inoculum of Thielaviopsis basicola.

Cultivar	Severity[x]
Judy's Pride	3.4 a
Burley 21	3.0 b
Ky 14	2.6 c
Ky 14 x 18	2.1 d
Ky 15	1.0 e
N. debneyi	1.0 e
Burley 49	1.0 e

[x]Average over 9 reps with 4 plants per rep. Severity on scale of 1-5 with 1=healthy and 5= >95% root rot.

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Table 3. Black root rot severity in relation to tobacco cultivar and fumigation in Buncombe County[X]

Cultivar	Root rot rating	
	Fumigated	Nonfumigated
Kentucky 14	2.3	4.0
Kentucky 14 x L-8	1.8	3.8
Burley 21	2.3	3.5
Tennessee 86	1.0	1.0

[X]Root rot severity determined one week after topping, 8/1/86. Severity on scale of 1-5 with 1=healthy and 5=> 95% root rot.

Table 4. Populations of Thielaviopsis basicola in five burley tobacco fields before and after fumigation.

County	Propagules/g soil		
	Nonfumigated	Fumigated	Reduction (%)
Mitchell	700	---	---
Buncombe	210	30	86
Haywood	180	110	40
Yancey	350	---	--
Madison	90	20	78

Table 5. Effect of fumigation on yield of burley tobacco in soil infested with Thielaviopsis basicola[X,Y]

Cultivar	Treatment/Yield (lbs. per acre)		
	Nonfumigated	Fumigated	% increase
Kentucky 14	2671	3158	18.2
KY 14 x L-8	3133	3173	4.5
Burley 21	2370	3152	33.0
Tennessee 86	3363	3600	7.0

[X]Mean population of T. basicola 210 p/g in nonfumigated and 30 p/g in fumigated rows.

[Y]Soil fumigated with 25 g.p.a. of Telone C-17 three weeks prior to transplant.

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Table 6. Effect of temperature on infection of flue-cured tobacco by basidiospores of Thantephorus cucumeris

Temperature (C) Day:Night	Number of lesions per leaf
22:16	1.6
26:20	37.6
30:24	0.6
34:28	0.0

Table 7. Effect of temperature on lesion development.

Temperature (C) Day:Night	% of lesions expanding[x]	Max. rate of increase/day[y] (mm)
22:16	10	1.5
26:20	10	2.8
30:24	35	3.3
34:28	10	1.5

[x]Twenty lesions at each temperature regime measured.

[y]Maximum increase in lesion diameter per day.

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

II. Graduate Students

R. J. Pulliam, M.S., Plant Pathology. "Infection and colonization of susceptible and resistant flue-cured tobacco by Phytophthora parasitica var. nicotianae."

K. Jones, Ph.D., Plant Pathology.

V. Shew, H. D. 1987. Effect of host resistance on spread of Phytophthora parasitica var. nicotianae and the subsequent development of tobacco black shank under field conditions. Phytopathology 77: (In press).

VII. Shew, H. D., and Main, C. E. 1986. Effect of temperature and misting regime on development of Rhizoctonia leaf spot of tobacco. Phytopathology 76:1119.

Shew, H. D., and Main, C. E. 1987. Rhizoctonia leaf spot of tobacco. 23rd Tobacco Worker's Conf., Baltimore, MD.

Shew, H. D., Shoemaker, P. B., and Rufty, R. C. 1987. Studies on black root rot of burley tobacco. 23rd Tobacco Worker's Conf., Baltimore, MD.

VIII. R. J. Pulliam, M.S., Plant Pathology, August, 1986.

IX. I gratefully acknowledge the assistance of the NCSU Phytotron staff in the Rhizoctonia leaf spot disease research, and the technical assistance of D. T. Glover, E. C. Gray, and S. Bhikhai.

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Title: NC09326 Effects of Water Stress on Expression of Host Resistance to a Phytophthora sp.

Project Leader: H. D. Shew

I. Summary of Research

The effects of quantitative host resistance on inoculum production by Phytophthora parasitica var. nicotianae (Ppn) on roots of flue-cured tobacco was determined in lab and field tests. Detached roots were placed in field soil maintained at desired matric potentials in controlled temperature chambers. Total inoculum production decreased as level of resistance to Ppn propagules per mg of root for the susceptible and the low, moderate, and high matric potential. Inoculum production also was delayed in resistant compared to susceptible cultivars. Averaged over all soil types and moisture regimes, reproduction in the resistant cultivar Speight G-28 was 65% of the total in the susceptible cultivar. Data are summarized in Table 1.

Microplot tests were conducted to determine the effect of water stress on rate of development of tobacco black shank. Water stress in mid-to-late season, flowering to harvest, resulted in an increase in disease compared to nonstressed plots for resistant cultivars but not for susceptible cultivars.

Additional studies also addressed the mechanism(s) of resistance in tobacco to Ppn. Similar levels of infection were obtained in a susceptible and moderately resistant cultivar at a given zoospore concentration. However, root rot severity was significantly greater in the susceptible cultivar at a given inoculum level. Attraction of zoospores to roots was similar in the two cultivars. Colonization and reproduction was slower in the resistant cultivar. Data are summarized in Table 2.

Motility of zoospores of Ppn was studied. A zoospore suspension was placed in glass beads of known diameter to determine the effect of contact stimuli on encystment of zoospores of Ppn. As particle size decreased, duration of zoospore motility was reduced, indicating that zoospores of Ppn respond to contact stimuli. Zoospore motility in two black shank suppressive and two black shank conducive soils was related to zoospore motility and, therefore, root infection. In one suppressive soil, zoospores lost motility within 0.5 hour, whereas in a conducive soil many zoospores were still motile after 3 hours. Zoospores were very active in 1% soil extract from the suppressive soil, and remained motile for at least 4 hours. The mechanism of suppression would, therefore, not appear to be biological in origin. Soil sterilization had no effect on motility in any of the soils.

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Table 1. Effect of host resistance on inoculum production of Phytophthora parasitica var. nicotianae.

Cultivar	Propagules per mg root		% of susceptible	
	Run 1	Run 2	Run 1	Run 2
Hicks	4.1 a	9.1	--	--
Coker 319	3.4 ab	9.0	84	99
Coker 411	2.8 b	6.9	70	76
Speight G-28	2.7 b	5.8	65	64
NC 82		7.0		78

Mean of 4 reps, 4 root systems per rep.

Table 2. Infection incidence and disease severity on a black shank-resistant and flue-cured tobacco cultivar 'Hicks' and a black shank-resistant cultivar 'Speight G-28', inoculated by different zoospore concentrations of Phytophthora parasitica var. nicotianae.

Cultivar	Zoospores per plant	Infection[x] (%)	Severity[y] rating
Hicks	0	0	0.0
	10^2	42	0.8
	10^4	75	1.3
	10^5	96	2.3
	10^6	100	3.6
Speight G-28	0	0	0.0
	10^2	27	0.2
	10^4	69	0.2
	10^5	81	1.0
	10^6	100	2.0

[x]Infection determined by recovery of Phytophthora parasitica var. nicotianae from root systems plated on selective medium.

[y]Disease severity rating: 0=healthy root system and stem, 1= necrosis and thinning of the root system, 2=very thin and necrotic root systems and some stem lesions, 3=general crown and root rot, and 4=dead plant.

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IX. I gratefully acknowledge the technical assistance of D. T. Glover, E. C. Gray, and S. Bhikhai in this research.

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BIOLOGICAL CONTROL OF CERTAIN FOLIAR PLANT DISEASES AND NEMATODES IN TOBACCO

Harvey W. Spurr, Jr.

Lead Scientist, United States Department of Agriculture, Agricultural Research Service; Professor of Plant Pathology, N. C. State University
State 05615

I. Summary of Research:

This project is concerned with disease control mechanisms and their application to achieve plant disease control in a practical and economical manner. The emphasis is on new approaches and new techniques for understanding and efficiently controlling diseases. Specific objectives of immediate importance are: determine how to use leaf microflora to control foliar disease, determine how to induce host resistance to disease and develop practical applications, determine how nematodes survive during periods of stress, dormancy and anhydrobiosis.

Research during 1986 was highlighted by the results described in the following paragraphs.

Tobacco blue mold: A series of biochemical interactions between the tobacco leaf and fungal spores appear necessary to the development of a functional relationship between host and pathogen. Our first objective is to identify the biochemical components necessary for the specific interaction of P. tabacina with tobacco necessary to colonization by the fungus. P. tabacina spores were found to adhere to host and non-host leaf surfaces and inert surfaces. Leaf surface chemicals and cuticle tissue were isolated and are being tested to determine their effect on spore germination. The formation of appressoria by P. tabacina germ tubes is unknown. Research on appressorial structures has been initiated. Tobacco protein extracts and tobacco protoplasts are being tested for the presence of lectins. Our second objective is to determine how high humidity induces sporulation of P. tabacina in leaves. Experiments to determine the biochemical pathway(s) activated by high humidity are being initiated in a recently purchased dew chamber. (Cooperators: Mary L. Menetrez, D. A. Danehower)

The genetic composition of the fungal pathogen, Peronospora tabacina, determines its response to the environment. Therefore, with this obligate pathogen, genetic composition is the principal determinant of survival. Three studies are directed at determining this genetic composition and how it functions. The recent progress in these studies is summarized herein.

1. The Development of the Multinucleate Condition of Sporangia. One nucleus was observed to migrate into the developing sporangium. Additional migrations were prevented by the formation of a septum. This initial nucleus divided and then the daughter nuclei divided

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synchronously once or twice followed by random divisions. The resulting sporangia contained on average 15 nuclei with a range from 4 to 35 nuclei. This indicates that most sporangia are homokaryotic. Therefore, they may be less adaptable to new environmental conditions than heterokaryotic fungi.

2. Morphology and Distribution of Oospores. The function and relative significance of the sexual cycle needs to be determined. Oospores of two distinct morphological types were observed in diseased tobacco tissues. Samples of diseased tissue are being collected, fixed and examined for oospores from as many locations as possible. This should give information on where and to what extent genetic recombination is occurring.

3. World Collection/Pathogen Variability Study. A world collection of isolates of P. tabacina is being assembled at the Plant Disease Laboratory, Ft. Detrick, MD. This is the only approved containment facility in the U. S. and the personnel have much experience with downy mildews. Once the collection is assembled pathogenic variability and isozyme studies will be made. Recently, eleven isolates of Peronospora tabacina were collected temporally and spatially from populations in the Southern United States. The genetic variability of this collection was examined using starch gel electrophoresis. Sporangia of each isolate were screened for the presence of 32 enzymes of which 20 were found. Analysis of the enzyme bands indicated little variability existed between populations. The lack of variability may be due to the absence or failure of sexual recombination within the pathogen population and/or the inability of oospores that are produced to germinate and infect. This may explain the absence of metalxyl resistant isolates in this region whereas such isolates have been observed in other areas. Heterozygous loci coding for adenylate kinase, isocitrate dehydrogenase, and glucosephosphate isomerase were observed. This indicates the chromosome number of the asexual cycle of P. tabacina is either diploid (2N) or dikaryotic (N+N), a previously unknown fact. Since genetic diversity was identified at the allelic level, isozyme analysis was a valuable tool for assessing variability of the pathogen. (Cooperators: C. E. Main, M. R. Bonde, R. N. Trigiano, M. A. Moss)

Biological control of plant pathogens: A computer simulation model was developed to predict disease progression of Cercospora leafspot of peanut. The model was derived in part from an advisory system used for fungicide scheduling in North Carolina and Virginia. Basic infection rate was modeled as a function of hours of relative humidity >95%, minimum temperature during the period of high humidity, amount of infectious tissue, and proportion of uninfected tissue remaining. Latent and infectious periods were treated as distributed delay processes, and host plant growth (increase in leaflet number) was described as a logistic process. The model was validated using independent weather and disease severity data from field trials with peanut cultivar Florigiant, in which C. arachidicola was the predominant pathogen. Simulated disease progress curves and periods of rapid disease increase were similar to those observed in field trials. The model correctly ranked four epidemics in terms of end-of-season disease severity and area under the disease progress curve.

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A sub-model describing persistence and efficacy of chlorothalonil fungicide was incorporated into a computer simulation model of Cercospora leafspot of peanut. The resultant model was validated using independent data sets from field trials over a two-year period. Predicted disease progress curves and area under the disease progress curve for different fungicide application schedules and rates were compared with field observations. The model was then used to compare predicted disease severity and area under the disease progress curve for a calendar spray schedule vs a leafspot advisory program under different weather conditions. Predicted disease severity levels and area under disease progress curves were similar for advisory and calendar spray schedules. Results were insensitive to changes in parameters describing fungicide persistence or efficacy. (Cooperator: G. R. Knudsen)

III. Postdoctoral Fellows:

Dr. Vernon J. Elliott, ARS-USDA Research Associate, Biological Control of Foliar Pathogens. Pilot Test Program.

Dr. Mary L. Menetrez, Postdoctoral Fellow, Host Specificity and Sporulation of Peronospora tabacina, the Blue Mold Pathogen, Sponsor: R. J. Reynolds Tobacco.

Dr. Wayne M. Thal, Research Affiliate USDA-ARS, Biological Control of Leafspot Diseases, Sponsor: Del Monte Corporation.

IV. Publications:

Mohapatra, S. C., Arcila, J. and Spurr, H. W., Jr. 1986. Alteration of germination and protein synthesis by disinfection of tobacco seeds. Tob. Sci. 30:66-68.

Trigiano, R. N. and Spurr, H. W., Jr. 1986. The development of the multinucleate condition of Peronospora tabacina sporangia. (Abstr.) Phytopathology 46:1111.

V. Manuscripts Accepted for Publication:

Trigiano, R. N. and Spurr, H. W., Jr. 1987. The development of the multinucleate condition of Peronospora tabacina sporangia. Mycologia (in press).

Knudsen, G. R. and Spurr, H. W., Jr. 1987. Management of bacterial populations for foliar disease biocontrol. CRC Press: (in press).

Knudsen, G. R., Spurr, H. W., Jr. and Johson, C. S. 1987. A computer simulation model for Cercospora Leafspot of Peanut. Phytopathology: (in press).

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Knudsen, G. R., Johnson, C. S. and Spurr, H. W., Jr. 1987. Use of a simulation model to explore fungicide strategies for control of Cercospora leafspot of peanut. Peanut Sci. (in press)

Knudsen, G. R. and Spurr, H. W., Jr. 1987. Field survival and efficacy of five bacterial preparations for control of peanut leafspot. Plant Disease: (in press)

VI. Manuscripts in Review:

Moss, M. A., Bonde, M. R., Spurr, H. W., Jr., Main, C. E. and Peterson, G. L. 1987. Isozyme analysis of population variability of Peronospora tabacina in the United States. Phytopathology:

VII. Papers Presented at Professional Meetings:

Trigiano, R. N. and Spurr, H. W., Jr. The development of the multinucleate condition of Peronospora tabacina sporangia. Ann. Meeting, Am. Phytopathological Soc. Orlando, FL, Aug. 1986.

Spurr, H. W., Jr., Trigiano, R. N., Moss, M. A., Main, C. E. Genetic composition of the blue mold pathogen. 32nd Tobacco Workers Conf., Baltimore, MD, Jan. 1987.

Menetrez, M. L. and Spurr, H. W., Jr. Specificity of the blue mold pathogen. 32nd Tobacco Workers Conf. Baltimore, MD, Jan. 1987.

Spurr, H. W., Jr. Bacterization of tobacco transplants for disease control (Granville Wilt). 32nd Tobacco Workers Conf. Baltimore, MD, Jan. 1987.

IX. Acknowledgements:

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Title: NC 03791 Epidemiology and Control of Foliar Disease of Tobacco

Project Leader: C. E. Main

I. Summary of Research:

This project has the following objectives:

A. Monitor occurrence, distribution, and importance of foliar diseases of tobacco in North Carolina.

B. Determine the source(s) of primary inoculum of Peronospora tabacina.

C. Develop improved control tactics for foliar diseases using fungicides, resistant cultivars, and cultural practices.

D. Monitor the presence of ecotypes, strains and races of foliar pathogens.

E. Relate development and spread of foliar diseases to weather and climate.

F. Assess the impact of foliar diseases on yield and value.

G. Establish cooperative efforts on foliar diseases with state, federal, and international research centers.

STUDIES ON THE BLUE MOLD DISEASE OF TOBACCO

Blue mold, caused by the obligate fungus pathogen Peronospora tabacina, continues to be a serious disease threat to the tobacco industry in the United States and Latin America. Macroscale, explosive epidemics generally occur when the growing tobacco crop, the pathogen, and conducive weather conditions co-exist. During 1986-87, scientists at North Carolina State University and the USDA Tobacco Research Laboratory at Oxford, NC studied the biology, epidemiology, biometeorology, development of resistant cultivars, fungicidal control strategy, and fungicide residues using both flue-cured and burley tobaccos.

The objectives of the blue mold project are comprehensive and broad based involving: (1) applied approaches to breeding resistant varieties, formulating fungicidal control strategies and educating the tobacco growers, (2) basic investigations into the genetics and physiology of host resistance, (3) ecologic and genetic variability within the pathogen population, (4) biometeorological aspects of inoculum movement and disease spread, and (5) development of an education and contingency control program in the event a metalaxyl resistant strain of P. tabacina should be introduced into the United States.

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Twelve field experiments were conducted in North Carolina, 11 field tests in Puerto Rico and 6 field tests at four separate locations in Mexico. Blue mold occurred in September in Yancey County, NC but did not develop or spread further. Therefore, the North Carolina tests were used to evaluate the type, yield and quality of blue mold resistant breeding lines and to conduct fungicide residue analyses on cured-leaf. Valuable disease evaluations were obtained in the winter nursery tests at Gurabo, Puerto Rico, and on both the east and west coast of Mexico.

BIOLOGY AND PATHOGEN VARIATION

Efforts to develop monogenic host resistance to blue mold in Europe and Australia have met with limited success because of natural variation in pathogenecity and adaptability within the P. tabacina population. Ecologic (temperature tolerance) and fungicide resistant strains have been documented in North and Central America. A cooperative, in-depth study of pathogen variation was initiated in January, 1987 with the USDA Foreign Disease and Weed Laboratory, Frederick, Maryland. Such information on spore morphology, CORESTA response, isoenzyme/genetic differences, etc. will help us prepare for the risks our growers face. Also, the success of our host resistance and chemical control programs at NCSU will depend upon a better understanding of the variation within the pathogen population.

Eleven isolates of P. tabacina were collected between 1979-1986 from diverse populations in the southern United States. The potential for genetic variability within the collection was examined using starch gel electrophoresis. Sporangia of each isolate were produced and screened for the presence of 32 enzymes of which 20 were positively identified. Similarity of the enzyme bands indicated little variability between the populations. The apparent lack of genetic variability may be due to the absence or failure of sexual recombination within the pathogen population and/or the inability of oospores that are produced to germinate. This could help explain the absence of indigenous metalaxyl resistant strains in Southeast whereas such strains have been observed in tropical overwintering areas where the oospore is apparently functional. Seventeen enzymes coded as having homozygous loci. Heterozygous loci coding for adenylate kinase, isocitrate dehydrogenase, and glucosephosphate isomerase were observed. This indicates that chromosome number of sporangia produced during asexual cycle of P. tabacina is either diploid (2N) or dikaryotic (N+N). Genetic diversity was identified at the allelic level in this study, therefore isozyme analysis could prove to be a valuable tool for assessing pathogenic variability among pathogen populations.

To investigate the above hypothesis further, we are establishing a world collection of P. tabacina. Live isolates from as many countries as possible will be collected and transported to containment facilities at Frederick, MD for storage and further study. The necessary quarantine permits have been obtained and collection/shipping protocols have been formulated. A list of potential collaborators in Europe, the Mediterranean, North and Central America and Australia is being developed.

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The first set of exotic isolates were collected on the east coast of Mexico in February, 1987 where metalaxyl resistance and an apparent variation in systemic blue mold exists. This effort will continue and intensify in 1987-88.

The development of the multinucleate condition of asexual sporangia is being investigated. One nucelus was observed to migrate into the developing sporangium. Additional migrations were prevented by the formation of a septum. This initial nucleus divided and then the daughter nuclei divided synchronously once or twice followed by random divisions. The resulting sporangia contained on average 15 nuclei with a range from 4 to 35 nuclei. This indicates that most sporangia are homokaryotic. Therefore, they may be less adaptable to new environmental conditions than other heterokaryotic fungi. The function and relative significance of the sexual cycle needs to be determined. Oospores of two distinct morphological types have been observed in diseased tobacco tissues. Samples of diseased tissue are being collected, fixed and examined for oospores from as many US and other locations as possible. This should give further evidence of where and to what extent genetic recombination might be occurring.

In a separate but related study, we have investigated worldwide pathogenic variability using the CORESTA blue mold trap collection results from 1978-1980. This collection of eight cultivars (differentials) contains the known sources of resistant germplasm to blue mold and was evaluated in 59 separate experiments representing 24 countries in Europe, North and Central America during this nine year period. Multivariate analysis of this large data set indicates ecologic and pathogenic variability probably exists and can be partitioned into at least seven temporal and/or spatial patterns (clusters) representing the world distribution of P. tabacina. Tests conducted in North and Central America fell into only two of the seven potential pathogenecity/response clusters.

BIOCHEMISTRY AND PHYSIOLOGY (Mary Menetrez, Post-Doctorate, Oxford, NC)

A series of biochemical interactions between the tobacco leaf and fungal spores appear necessary to the development of a functional relationship between host and pathogen. Our first objective is to identify the biochemical components necessary for the specific intereaction of P. tabacina with tobacco necessary to colonization by the fungus. P. tabacina spores were found to adhere to host and non-host leaf surface and inert surfaces. Leaf surface chemicals and cuticle tissue were isolated and are being tested to determine their effect on spore germination. The formation of appressoria by P. tabacina germ tubes is unknown. Research on appressorial structures has been initiated. Tobacco protein extracts and tobacco protoplasts are being tested for the presence of lectins. Our second objective is to determine how humidity levels influence sporulation of P. tabacina in vivo. Experiments to determine the biochemical pathway(s) activated by high humidities are being initiated in a recently purchased dew chamber.

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EPIDEMIOLOGY AND BIOMETEOROLOGY

Biometeorological studies have focused on long-distance, airborne movement of inoculum (asexual fungus spores) necessary for new disease outbreaks each year and continued spread of the epidemic. A computer-assisted mapping technique, i.e. meteorological trajectory analysis, is being developed at NCSU that permits the tracking and documentation of probable inoculum pathways and identification of source regions. The SE United States, SW Texas and Mexico are all known or suspected sources of inoculum for North Carolina.

Long-range transport modeling for 1981 and 1982 occurrences in west-central Kentucky supported the hypothesis that native Nicotiana repanda populations in south-central Texas serves as the inoculum source. During the 1985 blue mold epidemic in Kentucky, first occurrences were reported in a north-south orientation stretching from Monroe County on the Tennessee border to Mercer County located in central Kentucky. The inoculum is thought to have arrived via meteorological trajectory, on or about May 27 from Texas. Corresponding radar composite charts show the presence of frequent shower and thunderstorm activity during this period and during early June. The north-to-south pattern of first occurrences seem to indicate wet deposition of spores in conjunction with one of these passing line of showers. Additional work is necessary to substantiate this scenario. A similar analysis will be conducted on the 1986 epidemic when the necessary meteorological data tapes become available from the NCDC in Asheville, NC.

Several new transport models are being examined. The Branching Atmospheric Trajectory (BAT) model provides a much needed refinement in modeling the diurnal changes in the structure of the Atmospheric Boundary Layer (ABL), within which inoculum is both released and transported. During the daytime, the ABL is a well-mixed layer 1 to 2 km thick. Spores released into this layer are soon mixed throughout. At night a surface-based inversion is formed, and the efficient vertical exchange processes characteristic of the daytime ABL are absent. The noctural ABL is often marked by dramatic changes in horizontal wind speed and direction with height. Thus spores located within the ground-based inversion are likely to be transported in a different direction and at a different speed than those above the inversion. This model is now fully operational at NCSU and is being used to examine both historic and new transport case studies providing a more realistic picture of the diurnal variations occurring in the lower atmosphere. Recently, the MESOPUFF II model has been acquired which incorporates an enhanced meteorological data base. This allows for greater spatial and temporal resolution of the lower atmosphere where the spores actually travel. A study of blue mold risk climatology analysis was initiated to integrate, in a probabilistic sense, the spatial and temporal fields of temperature, moisture and general atmospheric features in order to identify geographic areas at high risk to blue mold. This is a relatively new and long term effort.

The blue mold pathogen, P. tabacina, is thought to overwinter in SC

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Texas thereby providing inoculum in the spring and early summer as the N. repanda populations develop naturally from seed. A pilot study has been initiated, under a Memorandum-of-Agreement with Southwest Texas State University, to more accurately assess the strength of this inoculum source and the characteristics of the indigenous blue mold fungus in Texas. During 1987 we will work with botanists from Texas who are familiar with the habitat of N. repanda, to obtain quantitative survey data on the distribution and population densities of this species. Using such data, it is hoped to make a reasonable estimate of inoculum source strength (infected plants) which will become a necessary input into future trajectory analyses and possible forecasting programs.

BREEDING AND GENETICS (Rebeca Rufty and E. A. Wernsman, Crop Science)

The objectives of the plant breeding studies were to: (1) evaluate blue mold resistant flue-cured and burley germplasms for improved agronomic traits, chemical composition and performance, (2) determine the usability of the germplasm as a source of resistance, and (3) identify and characterize additional and/or new sources of blue mold resistance which may be utilized in the development of breeding lines. The long-term goal is to develop tobacco genotypes possessing multigenic resistance mechanisms thereby increasing the quantitative level of resistance. Such quantitative resistance may be more stable to pathogen variation and less vulnerable to population shifts in P. tabacina.

Seventy-five burley lines in the F_3 generation, derived from crosses of Ovens 62 x Ky 17 and Ovens 62 x Ky 15, were evaluated for resistance under severe blue mold pressure at Gurabo, Puerto Rico. Ten lines exhibited good resistance and were selected for further testing in 1987-88. Two experiments were conducted at Gurabo, P.R. to compare the level of resistance of flue-cured and burley breeding lines and F_1 hybrids possessing the Chemical Mutant resistance mechanism in both the homozygous and heteroyzgous state. All resistant lines and F_1 hybrids had significantly less leaf damage than susceptible Speight G-28. Resistant lines had lower (but not significant) disease than the hybrids. No significant differences were found among the resistant lines nor among the resistant F_1 hybrids. In a separate test four burley phenotype lines that were selected from a 1985-86 F_2 population of Ky 17 x Chemical Mutant, were advanced to the F_4 generation and hybridized with Ky 17. Susceptible Ky 17, the resistant lines and four F_1 hybrids were tested for blue mold reaction. Leaf damage was significantly lower on resistant lines and hyrids. Inbred lines had significantly less disease damage than the hybrids. The hybrids were intermediate in reaction between their parents. No significant variation was found among resistant lines nor among resistant hybrids. This study demonstrates that (1) individual plants and experimental lines with the Chemical Mutant resistance mechanism (but without the "feral" leaf phenotype) can be readily identified in segregating generations, and (2) under the conditions of the Gurabo test (environment, pathogen biotype, etc.) this resistance mechanism does not exhibit complete dominance as reported previously in the literature.

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In tests at Whiteville and Rocky Mount, NC agronomic performance, chemical composition, and usability evaluations were conducted on three blue mold resistant breeding lines, four susceptible cultivars and the F_1 hybrids between the resistant breeding lines and cultivars. Resistant breeding lines NC-BMR-42 and NC-BMR-62, doubled haploids between Ovens 62 x McNair 944, exhibited marked agronomic improvement over Ovens 62. The lines compared favorably with the susceptible cultivars for most characters. Although the grade index was lower for the resistant lines, indicating inferior leaf quality, the lines are considered highly usable. F_1 hybrids exhibited significant heterosis and high yields. Concentrations of total alkaloids and reducing sugars in the resistant lines and their hybrids appeared satisfactory.

Fungicide resistant strains of P. tabacina are known to exist in Mexico, Latin America and Cuba. Thirty-five flue-cured and burley lines with resistance to blue mold, plus appropriate susceptible checks, were planted at two locations in Mexico (Papantla & San Andres in the state of Veracruz on the east coast) where the metalaxyl resistant strain is present. Blue mold reaction and entry resistance rankings were identical to those obtained in Puerto Rico since 1983. Therefore, the resistant germplasm is expected to provide protection in the eventuality that metalaxyl resistant strains of P. tabacina are introduced into the United States.

The N. C. Breeders Release Board has recommended the release of NC-BMR-42 and NC-BMR-90 in 1987. Because of adequate resistance to blue mold and acceptable agronomic characteristics, NC-BMR-42 represents the best germplasm source available in the United States for development of flue-cured resistant cultivars. NC-BMR-90, with 50% burley tobacco germplasm, may be used by burley breeders. Presently there are no acceptable burley cultivars with blue mold resistance.

In controlled environment PHYTOTRON experiments conducted during the winter of 1986-87, the components of partial resistance in NC-BMR-42 and NC-BMR-90 are being identified and quantified. Compared with susceptible cultivars McNair 944 and Speight G-70, the two new lines exhibit good resistance to infection, incubation period, latent period, lesion area and amount of sporulation. The original resistant parents, Ovens 62 and Chemical Mutant, are intermediate in response to all the components measured. This is strong evidence that pathogenicity and/or populational shifts in P. tabacina will not overcome these resistance mechanism(s) thereby making the lines, and subsequent cultivars, useful for many years.

FUNGICIDE EVALUATION AND STRATEGY

Replicated fungicide tests for blue mold control were conducted in three distinct burley areas in western North Carolina, one flue-cured site at Clayton, NC and at Gurabo, Puerto Rico. Blue mold did not develop in the North Carolina tests. In the fungicide screening test in Puerto Rico, percent leaf area damage (LAD) was 0, 0, 2.4, 5.7, 8.3 and 19.2 for burley tobacco treated with metalaxyl, oxadixyl, mancozeb, benalaxyl + mancozeb,

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benalaxyl and the control, respectively. In a separate fungicide strategy test, contact sprays initiated two weeks following transplanting resulted in significantly less disease than sprays delayed until after onset of disease (4.0 vs. 22.8 LAD). However, no differences were found between systemic fungicides initiated 2 weeks following transplanting and sprays initiated at onset of disease (4.8 vs. 3.0 LAD). Results of tests conducted in the winter of 1986-87 are still being analyzed but appear to be similar to the previous year's results with one notable exception. Aliette, a reportedly systemic fungicide, was not efficacious against blue mold in 1986-87. The tests in Puerto Rico provided us with important data on fungicides and alternative fungicide use strategies for blue mold control which were not obtainable in North Carolina because of the absence or unpredictability of blue mold occurrence.

Contingency plans were developed to monitor and test P. tabacina isolates in North Carolina for sensitivity to metalaxyl in the event blue mold developed on metalaxyl treated burley tobacco in 1986. However, no such reports were recorded. Educational information on blue mold was provided to growers in various newsletters, during Burley Day programs, and at burley field tours and field days.

Cured leaf samples from the burley and flue-cured fungicide tests were saved from all EBDC plots and from individual harvests of selected treatments for residue analysis of EBDC and ETU, a metabolite of EBDC. A study of EBDC and ETU residues on 1985-86 flue-cured tobacco tests at Clayton, NC was completed. Maneb and mancozeb were applied at a rate of 0.9 kg/380 L at 5 or 7-day spraying intervals beginning May 30, 1985 and ending July 15, 1985. With a 5-day spray interval, residues of EBDC and ETU in samples composited over stalk positions averaged 28 and 0.06 ppm, respectively, for maneb-treated plots compared to 48 and 0.25 ppm, respectively, for mancozeb-treated plots. When a spray intervals of 7 days was used, EBDC and ETU levels were 42 and 0.19 ppm from mancozeb-treated plots. For a reduced spray strategy with 5-day intervals and four applications of mancozeb beginning June 3 and ending June 17, residues were 18 and <0.05 ppm of EBDC and ETU, respectively.

MEXICAN PROJECT

On site visits were made to Mexican tobacco production areas and NCSU test sites in December 1986 and February 1987. In addition to evaluating the CORESTA collection and breeding line tests, NCSU personnel had the opportunity to observe first hand the efforts of TABACOS MEXICANOS (TABAMEX) in controlling blue mold. Generally, the fungicide program using 12 day Ridomil MZ-58 spray schedule supplemented with a 4 day interval Mancozeb treatment has proven successful even in the presence of the metalaxyl resistant strain of P. tabacina. However, under unpredictable, frequent, long-lasting or severe weather conditions (rain, cloudy and cool weather), the TABAMAX fungicide evaluation and spray program was inadequate. To successfully evaluate and compare relative efficacies of fungicides and application strategies, a standard test design at multiple locations with proper attention to appropriate checks,

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application technology and disease assessment would be most useful. We propose to design and conduct such standardized tests in North Carolina (3 locations), Kentucky (1 or more locations), Puerto Rico (1 location) and Mexico (4 locations) during the 1987-88 growing seasons.

ACKNOWLEDGEMENTS

Blue mold epidemics are complex biological phenomena that can result in serious economic impact to North Carolina growers and the tobacco industry. History has proven this to be the case. The blue mold program at North Carolina State University is committed to finding practical solutions that should lessen the risk to our farmers each year. At the same time, basic research on the nature of the host-pathogen-environment components of the epidemic are being investigated. The blue mold-tobacco system represents one of the most promising case studies for phytopathological research available today. exciting. The expertise and efforts of nine scientists are involved in the research described in this report.

J. M. Davis	Meteorology and Plant Pathology
Mary Menetrez	Post-doctorate, USDA, Oxford
N. T. Powell	Extension/Research, Plant Pathology, Flue-cured
Rebeca Rufty	Crop Science
T. J. Sheets	Pesticide Residue Research Laboratory
P. B. Shoemaker	Extension/Research, Plant Pathology, Burley
H. W. Spurr, Jr.	USDA Oxford, Plant Pathology
E. A. Wernsman	Crop Science
C. E. Main	Coordinator, Plant Pathology

The interest and support of the North Carolina Tobacco Foundation and the RJR Nabisco Company is gratefully acknowledged.

II. Graduate Students: None

III. Postdoctora Fellows: None

IV. Publications:

Davis, J. M., and C. E. Main. Applying atmospheric trajectory analysis to problems in epidemiology. Plant Disease 70:490-497.

Main, C. E., J. M. Davis, and M. A. Moss. 1986. The biometeorology of blue mold of tobacco: A case study in the epidemiology of the disease. pp. 453-472. IN: D. R. MacKenzie, C. Barfield, T. G. Kennedy, and R. D. Berger (eds.). Movement and Dispersal of Agriculturally Important Biotic Agents. Claitor's Publ. Co., Baton Rouge, LA. 611 p.

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Davis, J. M., C. E. Main, and W. C. Nesmith. 1986. The biometeorology of blue mold of tobacco. The evidence for long-range sporangiospore transport. pp. 473-498. IN: D. R. MacKenzie, C. Barfield, T. G. Kennedy, and R. D. Berger (eds.). Movement and Dispersal of Agriculturally Important Biotic Agents. Claitor's Publ. Co., Baton Rouge, LA. 611 p.

V. Manuscripts Accepted for Publication: None

VI. Manuscripts in Review:

Moss, M. A., and C. E. Main. Evidence of temperature tolerance in Peronospora tabacina. Phytopathology.

Moss, M. A., and C. E. Main. The effect of temperature on sporulation and viability of Peronospora tabacina isolates collected in the United States. Phytopathology.

Moss, M. A., and C. E. Main. Factors affecting systemic infection of tobacco by Peronospora tabacina. Phytopathology.

Moss, M. A., M. R. Bonde, H. W. Spurr, Jr., C. E. Main, and G. L. Peterson. Isozyme analysis of Peronospora tabacina in relation to population variability in the United States. Phytopathology.

Byrne, S. V., C. E. Main, and R. Delon. Comparison of Peronospora tabacina in Europe and North America using the CORESTA Trap Collection. Plant Disease.

VII. Papers Presented at Professional Meetings:

Davis, J. M., and C. E. Main. 1986. The New Branching Atmospheric Trajectory Model. Phytopathology (APS Ann. Meeting, Orlando, FL, August 10-14, 1986).

Shew, H. D., and C. E. Main. 1986. Effect of temperature and misting regime on development of Rhizoctonia leaf spot of tobacco. Phytopathology (APS Ann. Meeting, Orlando, FL., August 10-14, 1986).

The 32nd Tobacco Workers' Conference, Baltimore, MD, January 12-15, 1987:

Byrne, S. V., and C. E. Main. The North Carolina crop loss reporting system.

Shew, H. D., and C. E. Main. Rhizoctonia leaf spot.

Spurr, H. W., Jr., R. N. Trigiano, M. A. Moss, and C. E. Main. Genetic composition of the blue mold pathogen.

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Main, C. E. The Texas inoculum source - fact or fantasy.

Moss, M. A., and C. E. Main. Effect of environment on systemic blue mold.

Byrne, S. V., and C. E. Main. The CORESTA trap crop collection - an analysis.

Main, C. E. The Collaborative NCSU/TABAMEX blue mold research in Mexico.

Rufty, Rebeca, E. A. Wernsman, and C. E. Main. Evaluation of tobacco germplasm in Puerto Rico for resistance to blue mold.

Shoemaker, P. B., and C. E. Main. Fungicides for control of blue mold.

VIII. Graduate Student Thesis Completed During Reporting Period: None

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Title: N.
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I. Summary

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Title: N. C. 05507 Disease Control Strategies for Burley Tobacco and Vegetables in Western North Carolina

Project Leader: Paul B. Shoemaker

I. Summary of Research:

A. Alternative fungicides and strategies for blue mold control on burley tobacco (C. E. Main, cooperating).

Replicated tests for blue mold (Peronospora tabacina) were conducted in three distinct geographic areas in western North Carolina and at Gurabo, Puerto Rico. Blue mold failed to develop in the North Carolina tests. Results from the Gurabo test, completed in Spring 1986, were encouraging. In a fungicide screening test, percent leaf area damage (LAD) was 0, 0, 2.4, 5.7, 8.3 and 19.2 for burley tobacco treated with metalaxyl, oxadixyl, mancozeb, benalaxyl + mancozeb, benalaxyl and the control, respectively. In a fungicide strategy test, contact sprays initiated two weeks after transplanting resulted in significantly less disease than when sprays were delayed until disease onset (4.0 vs. 22.8 LAD). However, there was no difference when systemic fungicides were initiated 2 weeks after transplanting compared to starting at disease onset (4.8 vs. 3.0 LAD). Results of tests conducted in winter 1986-87 are still being analyzed but appear to be similar to the previous year's results, except that Aliette, a reportedly systemic fungicide was not efficacious against blue mold. The tests in Puerto Rico provide data on alternative fungicides and fungicide use strategies for blue mold control which has not been obtainable from recent tests in N. C.

B. Black root rot on burley tobacco, its distribution and importance (H. D. Shew, cooperating)

Tests were conducted in fields in five counties in which soil had been previously assayed for the BRR organism, Thielaviopsis basicola. Eight cultivars differing in their resistance to BRR were planted in both fumigated and non-fumigated soil to determine their reaction to the disease and their influence on soil levels of T. basicola. Preliminary analyses of results indicate that where initial levels (I_o) were 210, 180, and 90 propagules per gram, fumigation with Telone C-17 reduced inoculum levels 86, 40, and 78%, respectively. Soil inoculum levels of the black root rot organism increased 3.2, 1.3 and 0.9 fold following production of cvs. Judy's Pride (susceptible), KY 14 (moderate resistance) and KY 15 (high resistance), respectively. In fields with I_o = 208, 136 and 11, the root rot ratings for Judy's Pride were 3, 2 and 0.5, respectively, indicating correlation between initial inoculum and disease development. Yields were significantly influenced by both resistance and fumigation. For example, average per acre yields for Burley 21 (low resistance), KY 14 (moderate resistance) and TN 86 (high resistance were 2370, 2671 and 3363 pounds in non-fumigated soil and 3152, 3158 and 3600 pounds in fumigated soil, respectively. The use of resistance and fumigation apparently produces an additive response. These tests will be repeated in 1987 and will provide the basis for making grower recommendations for controlling this disease.

2000270109

Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

VII. Papers Presented at Professional Meetings:

Shoemaker, P. B. 1986. Evaluation of fungicides for the control of blue mold. Presented at the Terces Seminario de Intercambio de Conocimientos sobre el Tabaco, Tepic, Mexico, 7-10 Aug.

Shoemaker, P. B. and C. E. Main. 1987. Fungicides for blue mold. Talk presented at 32nd Tobacco Workers Conference, Baltimore, Maryland, 12-15 January.

Shew, H. D, P. B. Shoemaker and R. C. Rufty. 1987. Studies on black root rot on burley in North Carolina. Talk presented at 32nd Tobacco Workers Conference, Baltimore, Maryland, 12-15 January.

IX. Acknowledgements Financial support provided by the N. C. Tobacco Foundation and R. J. Reynolds Blue Mold Project funds is gratefully acknowledged.

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Title: Development of a Computer Simulation Model of the Tobacco Production System

Project Leader: S. M. Schneider

I. Summary of Research:

The soybean growth model, SOYGRO version 5.0, developed by Wilkerson et. al at the University of Florida, was chosen as the structural framework for the development of the tobacco model. SOYGRO is a phenology based program which runs on IBM-compatible microcomputers. Dr. Wilkerson is now a member of the Crop Science faculty at North Carolina State University and is supportive of this project. Field plots were established at the Oxford Tobacco Research Station during the summer of 1986. Twelve plots from each of two varieties, NC2326 and K-399, were sampled each week beginning May 21st and ending September 16th. The following measurements were made on each leaf; length, width, fresh weight, dry weight, and surface area. Green leaf punches were taken and bulked across four plants and three leaf positions. These were analyzed to determine total alkaloid content. The whole leaf samples were freeze-dried, bulked across four plants and three leaf positions, and ground. These samples will be analyzed for nitrogen and sugar levels. As leaves ripened, they were sampled, cured, and ground. These samples will also be analyzed for alkaloids and sugars. Air and soil temperatures were monitored continuously. Rainfall/irrigation was measured daily. The automatic weather station for collecting solar radiation has not yet been installed at Oxford. Solar radiation data from Raleigh will be used for this data set. These data will be used to look at correlations between environmental factors, plant growth measurements, and chemical concentrations over time. Additionally, these data will be used to develop and test the tobacco model.

II. Graduate Students:

Terry Wheeler, Ph. D. student in plant pathology

VII. Papers Presented at Professional Meetings:

Schneider, S. M. and H. Ferris. An iteratine procedure for determining life cycle parameters from stage frequency data. 1986. American Phytopathological Society Annual Meeting, Orlando, FL. Aug. 10-14, 1986.

IX. Acknowledgements:

Appreciation is expressed to A. P. Currin, Jr. for technical assistance.

2000270111

Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

NC 3901 PLANT BED AND FIELD MANAGEMENT

OF FLUE-CURED TOBACCO

G. S. Miner, Project Leader
J. W. Wade, Research Technician

I. Summary of Research:

Experiments were initiated to determine the effectiveness of Basamid, a granular fumigant, as an alternative to methyl bromide in the fumigation of tobacco plant beds. The objectives were to (1) determine if a lower rate of Basamid was as effective as the rate recommended by the manufacture for weed control, and (2) evaluate various means of sealing the fumigant in the soil. Treatments consisted of Basamid applied at 5 and 7 lb/100 square yards combined with sealing treatments consisting of covering with plastic(no water), watering and covering with plastic, watering only, and rolling the soil surface followed by watering. The water serves to activate the fumigant and to seal the soil surface to prevent loss of gas. A standard methyl bromide treatment was used as a control. Treatments were replicated 3 times on plots measuring 4 square yards at the Border Belt Tobacco Research Station and Central Crops Research Station. All treatments were fall applied. All plastic covers were removed two weeks before seeding in the spring, fertilized in the conventional manner and plots were left uncovered until seeding. After seeding, plots covered with Remay.

Due to lack of weed pressure, little information was obtained at the Border Belt Tobacco Research Station. However, weed pressure was high at Central Crops Research Station and evaluation of this preliminary experiment suggests the 5 lb rate was not as effective as the 7 lb rate in controling weeds. Covering treated areas with plastic (with or without water) was more effective than leaving treated areas uncovered. The combination of the 7 lb rate of Basamid with plastic (with or without water) was similar to methyl bromide fumigation for weed control but the 5 lb rate under plastic was inferior. At pulling time, there were no significant differences among treatments in the number of plants but those from plastic covered Basamid treatments were larger than those from uncovered treatments. The smaller plants on uncovered treatments were associated with the greater weed pressure that occurred.

Dr. Doug Worsham assisted in evaluating the degree of weed control obtained and a summary of weed species observed and their distribution among treatments is given in Table 1. For comparison, there were no weeds observed in methyl bromide treated plots.

The question remains whether better weed control under plastic covers was due to more effective fumigation or the

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prevention of weed seed infestation during the months between fumigating and seeding. The uncovered treatments were exposed during this period and weed seed could have been entered the treated areas. Work will be continued to answer this question and to further evaluate Basamid as an alternative to methyl bromide. By incorporating Basamid closer to seeding time, we may be able to eliminate the use of plastic covers prior to seeding.

Cooperative work was initiated with Dr. Ray Campbell, NCDA, to relate leaf concentrations of N to leaf quality. Treatments consisted of rates of N and various harvest dates ranging from early to late. Tissue concentrations of N by stalk position were monitored at two week intervals during the season and we are attempting to relate N concentrations at harvest to leaf quality. Preliminary findings are not available due to the time lag on leaf analyses.

Table 1. Distribution of surviving weeds among species within rates of Basamid and Basamid management schemes.

Weed Species	P		P + H2O		H2O		Roll + H2O	
	5	7	5	7	5	7	5	7
	- - - - - - - - - - #/yd2- - - - - - - - - -							
Total	22	9	32	9	89	69	79	43
	- - - - - - - - % Distribution- - - - - - - -							
Annual Ryegrass	Ø	Ø	Ø	Ø	2	Ø	2	Ø
Annual Sedge	18	Ø	14	Ø	5	9	5	4
Carpetweed	55	100	66	75	32	30	26	27
Corn Spurrey	Ø	Ø	Ø	Ø	18	24	12	10
Crabgrass	13	Ø	10	Ø	3	8	Ø	6
Goosegrass	14	Ø	10	Ø	1	4	1	Ø
Primrose	Ø	Ø	Ø	25	17	5	31	10
Erect Knotweed	Ø	Ø	Ø	Ø	10	4	12	31
Swine Cress	Ø	Ø	10	10	13	16	12	10

II. Graduate Students:

One M.S. Candidate
Two Ph.D. Candidates (co-chaired with Drs. Peedin Raper, respectively)

III. Post Doctoral Fellows: None.

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

IV. Publications:

1) Miner, G. S., G. V. Gooding, Jr., and J. M. Moore. 1986. Prevention of mechanical transmission of tobacco mosaic virus in tobacco plant beds. Tob. Sci. 30:25-29.

2) Vepraskas, M. J. and G. S. Miner. 1986. Effects of subsoiling and mechanical impedance on tobacco root growth. Soil Sci. Soc. Am. J. 50:423-427.

3) Vepraskas, M. J., G. S. Miner, and G. F. Peedin. Relationships of dense tillage pans, soil properties, and subsoiling to tobacco root growth. Soil Sci. Soc. Am. J. 50:1541-1546.

V. Manuscripts Accepted for Publication:

1) Miner, G. S., A. Z. Galindez, and M. R. Tucker. Response of flue-cured tobacco to foliar and soil applications of Mn. Tob. Sci.

2) Smith, W. D., G. F. Peedin, W. K. Collins, M. R. Tucker, G. S. Miner and E. J. Kamprath. Tobacco response to sulfur on soils differing in depth to the argillic horizon. Tob. Sci.

VI. Manuscripts in Review:

1) Vepraskas, M. J. and G. S. Miner. Effects of soil, root and rainfall factors on alkaloid production in tobacco. Tob. Sci.

VII. Papers Presented at Professional Meetings:

1) Miner, G. S. Tobacco fertility and soil test correlation. College Fertilizer Conference. Virginia Beach, VA. July 27-29, 1986.

2) Miner, G. S., A. Z. Galindez, and M. R. Tucker. Response of Flue-Cured tobacco to foliar and soil applications of Mn. 32nd Tobacco Workers Conference. Baltimore, Md. January 12-15, 1987.

3) Vepraskas, M. J. and G. S. Miner. Relationships of tobacco alkaloid contents to subsoiling, root abundance, rainfall, soil texture and nitrogen rate. 32nd Tobacco Workers Conference. Baltimore, Md. January 12-15, 1987.

VIII. Graduate Student Theses Completed During Reporting Period: None.

2000270114

Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Title: NC 03659 Environmental Determinants of Crop Growth and Soil Productivity

Project Leader: C. D. Raper, Jr.

I. Summary of Research:

One of our research objectives has been to determine the allocation patterns of nitrogen and dry matter among the various organs of tobacco plants. Based on a model that describes nitrogen uptake as regulated by the reciprocal balance between demand for carbon and nitrogen products within the organs, we postulated that as external nitrogen availability for by roots declines a greater restriction occurs for leaf initiation and expansion than for photosynthetic rates per unit leaf area. Because leaf growth requires more carbon than nitrogen, a reduction in leaf growth by nitrogen stress should result in a shift of carbon allocation from shoot to roots. Limited nitrogen availability thus can result in an initial increase in root growth and dry matter accumulation. To test this hypothesis, tobacco plants were grown in hydroponic culture in the Phytotron. One group of plants was grown for 28 days after transplanting in a complete nutrient solution containing 1 mM nitrate, a second group was grown in a solution containing no nitrogen, and a third group was initially grown for 10 days in the solution containing no nitrogen and then transferred to the solution containing 1.0 mM nitrate. Within the initial ten days of growth in the absence of external nitrogen, emergence and expansion of leaves had ceased but the net photosynthetic rate per unit leaf area had declined only to about 40 percent of that for leaves of plants exposed to 1.0 mM nitrate. Thus, as postulated, leaf growth was restricted to a greater extent than photosynthetic activity in response to nitrogen stress. Compared to the plants receiving nitrate, allocation of dry matter to leaves of stressed plants rapidly declined while allocation of dry matter to roots increased to produce an initial increase in absolute dry weight of roots of stressed plants. A redistribution of nitrogen from leaves to roots also was noted in response to the nitrogen stress. As nitrogen stress continued beyond the first ten days, total dry matter allocation to roots declined in response to the combination of reduced photosynthetic rate and leaf area. The redistribution of nitrogen from leaves to roots, however, continued to occur in stressed plants. When external nitrogen was resupplied to stressed plants after ten days, photosynthetic rates and leaf emergence were restored and allocation of dry matter and nitrogen to leaves and roots returned to proportions in nonstressed plants.

II. Graduate Students:

Tom C. Granato (Ph.D.)
June Taeg Lim (Ph.D.)
James W. Rideout (Ph.D)

III. Postdoctoral Fellows:

Dr. Leslie Tolley-Henry

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

IV. Publications:

Tolley-Henry, L., and C. D. Raper, Jr. 1986. Utilization of ammonium as a nitrogen source: Effects of ambient acidity on growth and nitrogen accumulation by soybean. Plant Physiol. 82:54-60.

Tolley-Henry, L., and C. D. Raper, Jr. 1986. Nitrogen and dry matter partitioning in soybean plants during onset of and recovery from nitrogen stress. Bot. Gaz. 147:392-399.

Tolley-Henry, L., and C. D. Raper, Jr. 1986. Expansion and photosynthesis of leaves of soybean plants during onset of and recovery from nitrogen stress. Bot. Gaz. 147:400-406.

VII. Papers Presented at Professional Meetings:

Tolley-Henry, L., and C. D. Raper, Jr. Nitrogen and photosynthate allocation during vegetative growth of tobacco plants in response to onset of and recovery from nitrogen stress. Tobacco Workers Conference, 14 January 1987, Baltimore, Maryland.

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Title: N 3827 Cultural, Nutritional and Varietal Investigations with Burley Tobacco

Project Leader: J. E. Shelton

I. Summary of Research:

This project has the following objectives:

A. To evaluate methods of distinguishing differences in P & K requirements of soils with differing chemical and physical characteristics.

B. To correlate information from objective 1 with yields and quality of burley over a wide range of soil P and K levels and soil management systems.

Burley tobacco is grown on a wide range of soil conditions in western North Carolina that vary in textural classes from the sandy loam bottom land soils to heavy clay upland soils varying in organic matter content. Although, chemically, these soils vary considerably and react to fertilizer amendments differently, current P and K recommendations are the same for all soils having a given level of extractable P and K. The cation exchange capacity (CEC) ranges from 3.0 or less to a high of 15.0 me/100g or more. Current K_2O recommendations would result in theoretical K saturations of the CEC from 2.5 to 16%. On low CEC soils these rates may be excessive and could result in K leaching classes with insufficient available K for late season growth and maturity. On high, CEC soils the current rates may be insufficient. This could account for the general observation that coarse textured bottom land soils grow taller tobacco but cured leaf yields are lower than shorter tobacco grown on clay type soils.

In 1986 sites were selected with CEC ranging from 6.5-8.9 me/100g. Potassium was applied to give a theoretical percentage saturation from 2.5 to 8.9. Burley tobacco, cv. Ky 14 at 3 locations and Tn 86 at 2 locations, was grown using soil test recommendations for P_2O_5 and 225 kg ha^{-1} N. Yields at the various locations ranged from 1930-3423 kg ha^{-1}. Table 1 shows the K saturation percentage at which maximum yields were obtained at the various CEC's. At the lowest CEC level of 6.5 me/100g a K saturation of 8.4% was required for maximum yields. Whereas, at the highest CEC level only a 5.7 percent K saturation was required. Since no low CEC soils were included in the field studies a greenhouse study was conducted using a sandy soil with a CEC of 3.2 me/100g and a clay loam soil with a CEC of 8.6 me/100g. The recommended K rate would have resulted in a 10.5% K saturation in the case of the low CEC soil and 4.4% for the high CEC soil. Rates of K equivalent to 0, 1/2, 1, or 1 1/2 times the recommended rates were applied to the two soils. Supplemental K was applied at a rate of 56 kg ha^{-1} as a topdressing, to all treatments, three weeks after transplanting. The results of the greenhouse test are shown in Table 2 and with the low CEC soil high saturation percentages did not result in maximum growth. However, when the low CEC soil was topdressed with K the yields were comparable to those achieved on the high CEC soil.

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It appears that current K recommendations are adequate for soils with medium CEC's but high CEC soils may require more K and low CEC soils may require supplemental K for maximum tobacco yields. Field evaluation on low (less than 5 me/100g) soils will be conducted in 1987.

VI. Manuscripts In Review: Departmental Review

Dept. Manuscript #39 - Effect of Row Soil Management System and Nitrogen Rate on Burley Tobacco Production.

VII. Papers Presented At Professional Meetings

Shelton, J. E. 1987. Potassium recommendations for Burley Tobacco grown on different soil types. The 32nd Tobacco Workers Conference January 1987, Baltimore, Maryland

2000270118

Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Table 1. Effect on burley tobacco yields of K rates based upon K saturation of CEC on 5 soils.

Soil CEC	% K Saturation for Maximum Yield
6.5	8.4
7.0	7.2
7.8	7.9
7.9	7.0
8.9	5.7

Table 2. Effect of K application rate on yield of greenhouse container grown burley tobacco.

Relative Rate (RR)	Soil A*		Soil B	
	RR	RR+TD	RR	RR+TD
	g DW/pot.			
0	21	42	31	38
1/2y	36	49	40	44
y	40	50	50	51
1-1/2y	43	52	55	50

*Soil Test	CEC	KI	K_2O Recommendation	1>%
Soil A	3.2	67	123 kg ha^{-1} K_2O	10.5
Soil B	8.6	75	100 kg ha^{-1} K_2O	4.4

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

IV.

Title: NC 3998 THE EFFECT OF TILLAGE AND COVER SPECIES ON NITROGEN MOVEMENT IN MOUNTAIN AGRICULTURAL SYSTEMS.

Project Leader: G. D. Hoyt

V.

I. Summary of Research:

This project has the following objectives:

A. To establish conservation tillage for burley tobacco production in the mountain region on soils subject to erosion.

B. To determine optimum fertilizer source and placement for conservation tilled burley tobacco.

C. To determine the effect of various tillage systems on tobacco growth, yield, and root development.

VI.

Various management systems were established in 1986 to further evaluate the consequences of conservation tillage and cover crop residues on tobacco production. Previous research concluded that rye or barley residue utilized with a conservation tillage program could provide competitive yields with conventional culture and provide needed reduction in erosion. Although yields are comparable at the end of a growing season, early transplant vigor is reduced when conservation tillage is applied. Current research now addresses this subject (vigor reduction) by various fertilizer schemes for supplying adequate nutrition early in the season. The first experiment was established using two nitrogen sources (ammonium sulfate and sodium nitrate) that were broadcast throughout the plot and then left on the surface or incorporated in the strip-tilled area. Neither the source of N fertilizer nor the placement affected tobacco flowering during the season. Treatment also did not give a statistical increase in tobacco growth during early plant stages, and only the zero N fertilizer treatment produced yields significantly different from the other four treatments at harvest (Tables 1 and 2).

VII.

In the second experiment three tillage systems were compared using conventional tillage, strip-tillage, and no-tillage treatments. Flowering data showed little difference in early season growth among the three tillage treatments, and although final leaf yields are not significantly different, conventional tillage yielded higher than the other two forms of conservation tillage (1985 yield results show this same trend).

IV. Publications

XI.

Hoyt, G. D. 1987. Conservation tillage for burley tobacco, pp 22-24. In: 1987 Burley Tobacco Information. North Carolina Agricultural Extension. Publ. AG 376.

Hoyt, G. D. and W. L. Hargrove. 1986. Legume cover crops for improving crop and soil management in the southern U. S. HortScience. 21:397-402.

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

IV. Publications (continued)

Hoyt, G. D. and T. R. Konsler. 1986. Conservation Tillage for Vegetables and Tobacco, pp. 83-84. In: No-tillage Research Update - North Carolina, A. D. Worsham, ed., Proc. of the Southern Region No-tillage Conf., R. E. Phillips, ed., Southern Region Series Bulletin 319. pp. 127.

V. Manuscripts accepted

Hoyt, G. D. 1987. Legumes as a green manure for conservation tillage. In: The Role of Legumes in Conservation Tillage Systems. Soil Conservation Society of America Publication. In press.

Gilliam, J. W. and G. D. Hoyt. 1987. Effect of conservation tillage on fate and transport of nitrogen. In: T. J. Logan, J. M. Davidson, and M. R. Overcash (eds). Effects of Conservation Tillage on Ground Water Quality. Lewis Publishers, Inc., Chelsea, MI. In press.

VI. Manuscripts in review

Hoyt, G. D. and A. D. Cole. 1987. Soil nitrogen movement and transformation in conservation tilled tobacco. Soil Sci. Soc. North Carolina Proc. 30: In review.

VII. Papers presented at professional meetings

Soil nitrogen movement and transformation in conservation-tilled tobacco. G. D. Hoyt. Presented before the N. C. Soil Sci. Soc. Annual Meeting Raleigh, January, 1987.

Soil nitrogen movement and transformation in conservation-tilled tobacco. G. D. Hoyt. Presented before the 32nd Tobacco Workers Conference, January, 1987.

Conservation Tillage Systems for Vegetable and Tobacco Production. G. D. Hoyt and T. R. Konsler. Presented before the Soil Conservation Society Annual Meeting, Winston-Salem, NC, August, 1986.

Using Green Manure Crops: Which Ones are Best? G. D. Hoyt. Presented before the N. C. Alternative Farming Field Day. Raleigh, June, 1986.

Effect of conservation tillage on fate and transport of nitrogen. J. W. Gilliam and G. D. Hoyt. Presented before the Conservation Tillage Workshop, Chicago, IL, June, 1986.

XI. Acknowledgements

I wish to thank Mr. Anthony Cole for his excellent technical assistance on this project.

2000270121

Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Table 1. The effect of nitrogen source and placement on tobacco flowering.

Treatment		Plants Flowering									
Fertilizer Source	Placement	73*	78	80	83	84	87	89	92	96	98
		%									
0		4	6	7	13	17	21	21	34	44	50
NO_3-N	Surface	8	13	14	15	15	24	32	37	43	55
NO_3-N	Incorporated	0	3	5	11	14	19	21	28	40	50
NH_4-N	Surface	2	11	13	20	20	25	30	46	52	61
NH_4-N	Incorporated	2	11	15	21	22	31	35	38	47	52
LSD (.05)		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Days after transplanting

Table 2. The effect of nitrogen source and placement on tobacco growth and yield.

Treatment		Preharvest Sample			Harvest
Fertilizer Source	Placement	36*	57	84	118
		g/plant			kg/ha
0		7.25	35.5	103	1467
NO_3-N	Surface	9.00	41.0	164	2748
NO_3-N	Incorporated	10.00	47.0	134	2364
NH_4-N	Surface	8.63	42.5	168	2183
NH_4-N	Incorporated	10.50	52.0	145	2572
LSD (.05)		NS	NS	NS	826

*Days after transplanting

Table 3. The effect of tillage on tobacco flowering and yield.

Treatment	Plants Flowering						Yield
	87*	89	92	96	98	101	kg/ha
	%						
Conventional	5	13	13	22	29	45	2683
Strip-tillage	4	6	16	24	29	35	2256
No-tillage	6	7	9	20	23	38	2436
LSD (.05)	NS	NS	NS	NS	NS	NS	NS

*Days after transplanting

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TOBACCO LITERATURE SERVICE

Annual Report for the Calendar Year 1986

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		1985	1986	Change
I.	TOBACCO ABSTRACTS.			
	Abstracts published	1,285	1,978	+693
	Publications searched	5,964	6,100	+136
	Average per month	497	508	+ 11
	Distribution			
	Domestic:			
	Exchange	49	49	Nc
	Complimentary	109	109	Nc
	Subscriptions	115	97	−18
	Total	273	255	−18
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	Exchange	144	141	−3
	Complimentary	27	27	Nc
	Subscriptions	152	137	−15
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	Total, All Categories	596	560	−36
	Copies Per Year	2,980	2,800	−180
II.	TOBACCO REPRINT SERIES.			
	Reprints published (#713-721)	14	8	−6
	Recipients	160	165	+5
	Copies distributed	2,240	1,320	−920
III.	BIBLIOGRAPHIC INFORMATION SERVICE			
	Requests served	1,100	1,100	Nc
IV.	PHOTOCOPY SERVICE.			
	Tobacco Staff	6,950p.	18,944p	+11,994
	Paid Orders	525p.	4,376p	+ 3,851
V.	OTHER PUBLICATIONS DISTRIBUTED.			
	"Recent Advances in Tobacco Science".	69	59	−10

2000270123

Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

ADDENDUM

Listed below, by department, are research activities which received supplemental funding from the North Carolina Tobacco Foundation, Inc. for the period July 1, 1986 through June 30, 1987.

Activity	Investigator(s)
Biological and Agricultural Engineering	
Mechanization in Production of Burley Tobacco	C. W. Suggs
Stress Physiology of Tobacco Seedling Development	S. C. Mohapatra
Measurement of Optical Properties of Tobacco Related to Texture, Body and Color	W. F. McClure R. E. Williamson F. G. Giesbrecht
Investigation of Alternative Means of Removing Moisture from Tobacco	C. F. Abrams, Jr. S. C. Mohapatra
Applications of Expert Systems to Tobacco Production Decisions	R. S. Sowell
Crop Science	
Moving the "NC E-Z" Tobacco Breeding Method to Commercial Application	E. A. Wernsman
Tobacco Analytical Service Laboratory	W. W. Weeks
Leaf Surface Chemistry of *Nicotiana*: Rapid Methods for the Analysis of Component Classes	D. A. Danehower
Genetic and Breeding Studies for Bacterial Wilt Resistance in Tobacco	G. R. Gwynn
Remobilization of Nitrogen During Growth and Senescence of Flue-Cured Tobacco	R. C. Long
Genetic Investigations on the Angular Leaf Spot Disease of Tobacco Caused by *Pseudomonas angulata*	R. C. Rufty E. A. Wernsman

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Activity	Investigator(s)
Crop Science (continued)	
Physiology of the Growth and Development of Burley Tobacco	R. C. Long
Genetic Studies of Resistance to Black Root Rot in Burley Tobacco	R. C. Rufty
No-Till Tobacco	A. D. Worsham R. L. Davis
Effects of Harvest Maturity and N Rate on Some Agronomic and Chemical Characteristics of Flue-Cured Tobacco	R. C. Long G. F. Peedin M. A. Tucker
Economics and Business	
Explanation of the Size and Structure in Tobacco Farming	D. A. Sumner
Study on Costs of Producing Flue-Cured Tobacco Leaf Quality	S. A. Hatchett
Economic Analysis of Tobacco Quality	P. R. Johnson
Entomology	
Soil Insects Associated with No-Tillage Practices	G. J. House D. P. Schmitt D. M. Jackson
Effects of Host-Plant Health in the Bionomics of the Green Peach Aphid	E. P. Lampert
Ovipositional Response of Tobacco Budworm Moths to *Nicotiana* Species	D. M. Jackson
Monitoring and Development of Improved Sampling Procedures for Establishing Insect Abundance in Tobacco	E. P. Lampert
Effects of Aphid Control on Incidence of Viruses in Large-Field Plots	E. P. Lampert G. V. Gooding, Jr.

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Source: https://www.industrydocuments.ucsf.edu/docs/zsyk0000

Activity	Investigator(s)
Entomology (Pesticide Residue Research Laboratory)	
Residues of Prime+ on Flue-Cured Tobacco	T. J. Sheets H. Seltmann
Residues of Fenamiphos on Flue-Cured Tobacco	T. J. Sheets
Genetics	
Genetic Modification of Tobacco	D. F. Matzinger
Utilization of Recombinant DNA Technology to Explore Gene Regulation and Genetic Engineering in Tobacco	M. A. Conkling
Plant Pathology	
Biological Control and Genetic Studies of Bacterial Wilt in Tobacco	E. Echandi H. W. Spurr, Jr. G. R. Gwynn
Epidemiology of the Tobacco Black Shank Disease	H. D. Shew
Control of Flue-Cured Tobacco Diseases by Cultural Practices, Varietal Resistance and Chemical Applications	N. T. Powell
Breeding for Resistance to Tobacco Vein Mottling, Etch and Potato Virus Y	G. V. Gooding, Jr.
Influence of Host Resistance or Tolerance in Burley Tobacco on Viral Pathogenesis	J. W. Moyer G. V. Gooding, Jr. R. C. Rufty
Biology and Ecology of *Rhizoctonia solani* Causing Leaf Spot	H. D. Shew
Development of New Selection Schemes for Tobacco Protoplast Fusion	M. E. Daub
Development of Blue Mold Simulation/Management Model	C. E. Main
Development of Computer Simulation Model of Tobacco Production System	S. M. Schneider

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Activity	Investigator(s)
Plant Pathology (continued)	
Distribution of *Pseudomonas solanacearum* in Tobacco Soils	E. Echandi N. T. Powell H. D. Shew H. W. Spurr, Jr.
Alternative Fungicides and Strategies for Blue Mold Control on Burley Tobacco	P. B. Shoemaker
Study of Black Root Rot on Burley Tobacco	P. B. Shoemaker H. D. Shew
Soil Science	
Tobacco Root Distributions in No-Tilled and Conventionally Tilled Soils	M. J. Vepraskas G. D. Hoyt G. S. Miner
Tillage Systems for Burley Tobacco Production	G. D. Hoyt
Evaluate Methods of Determining Fertilizer Needs of Varying Soils in Production of Burley Tobacco	J. E. Shelton
Mechanisms of Flower Induction and Management Alternatives for Premature Flowering in Flue-Cured Tobacco	G. S. Miner C. D. Raper
General	
Director's Reserve for Emergencies and Reserve Research Assistantships	W. H. Johnson

Please keep in mind that the information presented in this annual report is NOT FOR PUBLICATION but merely represents preliminary findings which may later appear in published form. We trust that you will find the information presented herein of interest and value.

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